DESIGN AND DEVELOPMENT OF NOVEL TRANSDERMAL FORMULATION OF *CISSUS QUANDRAGULARIS* LINN. FOR THE MANAGEMENT OF ARTHRITIS AND BONE HEALING

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SRI BALAJI VIDYAPEETH UNIVERSITY

Deemed to be University (NAAC 'A^{++}') PUDUCHERRY – 607402, India

Submitted by

KIRUBA MOHANDOSS

Reg no. 1905002046

Under the guidance of

Dr. Vijayan V
Professor,
School of Pharmacy
Sri Balaji Vidyapeeth University, Puducherry

SRI BALAJI VIDYAPEETH

PUDUCHERRY

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ABSTRACT

The study aims to develop a transdermal *Cissus quadrangularis* phytosome-encapsulated patch to improve arthritis treatment and bone health, utilizing the herb's anti-inflammatory and bone-strengthening properties. The stem's distinct morphological characteristics were rectangular shaped, chloroplast-containing cortical cells, and discontinuous vascular bundles. Using Soxhlet extraction, crude ethanolic extracts (CQE) were obtained, resulting in a dark brownish-yellow colour, which was high in flavonoids, phenolic compounds, alkaloids, and other phytochemicals. Bioactive substances such as cholesterin, beta-sitosterol acetate, and quercetin-3-O-alpha-L-rhamnopyranoside were proven to be present using LC-MS, GC-MS, HPLC, and molecular docking. The *Cissus quadrangularis* phytosomes (CQE phytosomes) formulation showed improved stability and bioavailability. The CQE phytosomes exhibited noteworthy bioactivity as revealed by antioxidant tests and the biochemical assay demonstrates anti-inflammatory activity. The skin irritation study has proven the CQE phytosome's safety profile and tolerability. By utilizing the solvent evaporation process, the produced phytosomes were integrated into transdermal patches; formulation F2 is potential for therapeutic applications since it exhibited the highest drug content, low moisture content, thickness, flexibility, and in-vitro diffusion study demonstrated a controlled release which was Fickian diffusion. Three months of stability testing revealed no discernible changes in the F2 patch integrity, along with promising in vivo efficacy and safety profiles.

1. INTRODUCTION

Bones are vital for supporting the body, protecting organ systems, and regulating mineral and organic material concentration. They form through intramembranous and endochondral ossification during embryonic development. Bone remodelling occurs throughout life with osteoblasts secreting collagen and other organic components, which are mineralised, creating new bone tissue. Factors regulating bone formation include growth factors and hormones, influenced by mechanical forces like weight-bearing exercise.

Indigenous medicine, also known as traditional medicine, is a broad and diverse array of healing practices developed in specific regions, cultures, or countries. The Indian system of medicine is a significant form of traditional health care, incorporating medical viewpoints from Greece, Germany, and sacred scriptures. Traditional and Complementary Medicine (T&CM) is appreciated for its availability, affordability, cultural relevance, and critical importance to millions of people's trustworthiness.

Bone-related diseases (such as osteoporosis, osteoarthritis, and fractures) remain a significant health burden worldwide. Alternative medicine, particularly natural remedies in the form of phyto-molecules obtained from plants and animal products, is being explored for their potential in treating bone-related diseases. *Cissus quadrangularis*, an Indian native, is used worldwide to treat various conditions, including fractures [1,2]

A recent study on the use of this herb to alleviate inflammatory diseases and immunize bone and health highlights the need for efficient pain management and reduced treatment costs. A novel delivery system called phytosome has been developed to overcome the limitations of large compounds and low lipid solubility in plants. This system facilitates transdermal distribution, promoting bone health and healing of inflammatory areas. The study's goal is to create and test a phytosome-based mixture that will make transdermal *Cissus* patches work better for treating arthritis and improving bone health. This mixture will also improve the healing properties of *Cissus quadrangularis*.

2. AIM

This study aims to create and test a new transdermal patch containing *Cissus quadrangularis* phytosomes that can be used to treat arthritis and improve bone health.

OBJECTIVE

- **Extraction and characterization of bioactive components**: Extract and characterize the bioactive components from *Cissus quadrangularis* to ensure the formulation's yield and purity.
- **Synthesis and evaluation of phytosomes**: Synthesize phytosomes loaded with *Cissus quadrangularis* and evaluate their stability, effectiveness, and physical and chemical characteristics through in vitro experiments.
- **Development and assessment of transdermal patches**: Develop transdermal patches containing phytosomes from *Cissus quadrangularis* extracts and assess their effectiveness through both in-vitro and in-vivo investigations.

3. HYPOTHESIS

Transdermal delivery of *Cissus quadrangularis*, with its anti-inflammatory and bone-strengthening qualities, may help to control arthritis symptoms and enhance bone healing.

RESEARCH QUESTIONS

- What are the critical factors that optimise the transdermal delivery system of *Cissus quandragularis* Linn. for the effective treatment of arthritis?
- How does the effectiveness of this transdermal formulation compare with traditional oral forms in treating arthritis and promoting bone healing?
- What are the therapeutic effects of this novel transdermal formulation on arthritis management and bone healing?

4. REVIEW OF LITERATURE

4.1. *Cissus quandragularis*

- **Syend *et al*., 2022** [3] investigated the effects of ethanolic extract from *Cissus quandragularis* on diabetic cardiomyopathy in rats. The results showed that EECQ reduced hypercholesteremia, insulin intolerance, glucose levels, oxidative stress, heart inflammation hypertrophic biomarkers, and alleviated cardiac dysfunction.
- **Ondijo *et al*., 2022** [4] studied zinc oxide-activated carbon nanocomposite to remediate dieldrin in aqueous medium, with the nanocomposite outperforming activated carbon in sorbing capacity. The study suggests evaluating the nanocomposite's remediation capabilities for other persistent organic contaminants.
- **Bhat *et al*., 2022** [5] Click or tap here to enter text.evaluated a study on New Zealand white rabbits showed that *Cissus quandragularis* hydrogel, when coated with titanium implants, significantly improved the osseointegration of the implants, with the average mean values being higher than uncoated ones.
- **Narayana swamy *et al*., 2022** [6] analyzed the therapeutic properties of various stem extracts of the tropical herb *Cissus quadrangularis*, revealing a range of compounds, including carbohydrates, proteins, amino acids, and steroids, with varying extractive values.

4.2. Phytosomes

- **Kazemi *et al*., 2022** [7] developed a pomegranate peel product using a sustainable resin column chromatography process, enriched with punicalagin and encapsulated onto nano-phytosomes. The ratio of resin to extract increased phenolic compounds by 2.63-fold, achieving 58% encapsulation effectiveness.
- **Komeil *et al*., 2022** [8] researched surface-modified genistein phytosomes, containing hyaluronic acid and pegylated phospholipid for breast cancer treatment. These formulations

show promising chemotherapeutic effects, reducing tumour size and biomarkers and further research is possible for metastatic breast cancer.

- **Cicero *et al*., 2022** [9] conducted research involving 20 healthy young volunteers and found that acute CoQ10 phytosome supplementation improved endothelial responsiveness and overall antioxidant capacity, while middle-term treatment increased mean arterial pressure.
- **Pasala *et al*., 2022** [10] synthesized silybin phytosome (SIBP) and evaluated its effectiveness in protecting rats from cerebral ischemia-reperfusion injury. Results showed a doubled concentration of SIBP, increased glutathione and superoxide dismutase levels improved CA1 neuron organization and cortical cell death.

4.3. Transdermal patch

- **Shang *et al*., 2022** [11] investigated common causes of arthritis injuries including sports, aging, accidents, and illnesses. Transdermal delivery offers a safer, more effective approach, but its effectiveness is limited by the synovial capsule and skin barriers.
- **Zhang *et al*., 2022** [12] researched arthritic injuries that often lead to adverse events and transdermal delivery offers effective treatment. However, synovial capsules and skin barriers limit effectiveness. Nanomedicine-based formulations show safety and effectiveness, requiring further research.
- **Chen *et al*., 2022** [13] reported transdermal medication delivery through the skin is safe and effective, with transferosomes, and flexible vesicles, enhancing solubility and absorption of phosphatidylcholine. This mini-review highlights nanotechnology's potential for delivering bioactive phytochemicals.
- **Latif *et al*., 2021** [14] Click or tap here to enter text.developed a study on transdermal patches containing methotrexate and HPMC for treating localized psoriasis. The patches showed compatibility with medication and polymers, with F5 showing 36.55% cumulative CQE diffusion and 82.71% sustained release.

5. METHODOLOGY

5.1. Plant collection

The stems of *Cissus quandragularis* (Figure 1) were collected from Coimbatore, Tamil Nadu, India. To preserve their chemical components, the stems were dried in the shade. The plant material was authenticated by the Siddha Central Research Institute, PP Garden, Arumbakkam, Chennai, Tamil Nadu – 600106. Following authentication, macroscopic and microscopic examinations of the *Cissus quandragularis* stem were conducted and reported.

Figure 1: Stems of *Cissus quadrangularis*

5.2. Preparation of extract (hot continuous extraction)

Two kilograms of shade-dried *Cissus quandragularis* stems were crushed and packed into a filter paper thimble. Using a Soxhlet apparatus, the thimble containing 500 g of ground *Cissus quandragularis* was placed in the extraction chamber. Ethanol was used as the solvent and the extraction process was repeated until no trace of solvent remained, indicating complete extraction. The crude ethanolic extract was then stored for further studies.

5.3. Phytochemical evaluation

5.3.1. Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was employed to identify various phytochemicals present in *Cissus quadrangularis*. For flavonoids, quercetin was used as the standard with a mobile phase of n-butanol, ethyl acetate, and water (1:2:3). Alkaloids were identified using quinine as the standard, with ethyl acetate, chloroform, and water (5:3:1) as the mobile phase. Phenols were analysed using gallic acid as the standard and a methanol-water mixture (2:1) as the mobile phase. For tannins, gallic acid served as the standard, using chloroform and water (3:2) as the mobile phase.

5.3.2. LC-MS analysis

Liquid chromatography-mass spectrometry (LC-MS) was utilized to analyze the ethanolic extract of CQ. The system, calibrated at 340 nm, provided detailed molecular weight information and identified various chemical components.

5.3.3. GC-MS Analysis

Gas chromatography-mass spectrometry (GC-MS-thermo scientific MS DSQ II) identified and compared mass values of extract components.

5.3.4. HPLC Analysis

High-performance liquid chromatography (HPLC) further analyzed the ethanolic extract's chemical composition using gradient elution and a UV detector set at 210 nm.

5.3.5. Docking studies and molecular dynamics (MD) simulation

AutoDock Vina 4.2.6 facilitated docking studies to predict ligand binding efficiency with target proteins. The molecular dynamics (MD) simulations, conducted using Desmond 2020.1, equilibrated protein-ligand complexes and monitored stability through parameters like RMSD (Root mean square deviation), RMSF (Root mean square fluctuation), Rg (the radius of gyration), and SASA (Solvent Accessible Surface Area).

5.3.6. Binding free energy analysis

Binding free energies of ligand-protein complexes were calculated using the MM-GBSA (Molecular mechanics with Generalised Born and Surface Area Solvation) approach. The Python script thermal mmgbsa.py was used to estimate the binding free energy from simulation trajectories.

5.4. Formulation of phytosomes

The method for developing ***Cissus quadrangularis*** **phytosomes** (**CQE phytosomes**) included sonication, rotational evaporation, and dissolving lecithin P30 in methanol and chloroform. To ensure complete mixing and film formation, the ethanolic extract was added to the thin film and the mixture was spun for 45 minutes at 60°C. The mixture was then subjected to 20 minutes of sonication at 100 ± 5 rpm at temperatures between 10°C and 20°C to yield the required particle size and improve the phytosome's structural integrity. After that, the phytosomes were preserved for examination. For a transdermal formulation, the best phytosomes were chosen to provide optimal stability and efficacy (Table 1).

Table 1: Development trials of phytosomes

Formulation code	CQE	Lipoid P 30 (Lecithin)
F1	1 g	4 g
F2	1 g	5 g
F3	1 g	6 g

5.5. CQE phytosome characterization

Several characterization techniques, including light microscopy, FTIR spectrometry, X-ray diffraction, FE-SEM, HRTEM, and Zeta potential tests, were used to confirm the optimized phytosomal formulation. To evaluate the phytosome's absorbance spectra, UV-vis spectroscopy was employed. A Zetasizer Nano S90 was used to assess the phytosomes dispersion and particle size distribution.

To accurately evaluate the concentration of the *Cissus quandragularis* ethanolic extract (CQE), a calibration curve was developed using a UV spectrophotometric method and a graph was plotted.

5.6.Characterization of standard beta-sitosterol (merck pvt, india)

5.6.1. Uv-vis spectroscopy scanning

A beta-sitosterol solution (1 mg in 10 mL ethanol) was scanned from 200-800 nm using a UV-Vis spectrophotometer to identify the characteristic peak around 200-300 nm [15]

5.6.2. Calibration curve for the standard beta-sitosterol

A calibration curve for beta-sitosterol at 220 nm was created by measuring the absorbance of standard ethanol solutions (0-12 µg/mL) using a UV-Vis spectrophotometer. The unknown concentration of Cissus quadrangularis phytosomes was then determined by comparing its absorbance to the calibration curve.

5.7.Calibration curve of *cissus quadrangularis* phytosomes

To accurately evaluate the concentration of the *Cissus quandragularis* ethanolic extract (CQE), a calibration curve was developed using a UV spectrophotometric method and a graph was plotted.

5.7.1. FT-IR Spectroscopy

The functional groups in the polymer and the ethanolic extract were identified using FT-IR, which was also used to establish the compatibility analysis.

5.7.2. DSC

CQE phytosome melting point was measured using differential scanning calorimetry (DSC), which involved heating a sample at a steady state. Understanding the phytosome behaviour under various situations was made easier by the critical information this analysis offered regarding the formulation's physical characteristics and thermal stability.

5.7.3. CQE content

The predefined quantity of phytosomes was dissolved in a buffer solution and the amount of CQ extract was determined using UV-Vis spectroscopy at a wavelength of 220 nm. Three runs of the experiment were conducted and the findings are presented as ±SD.

5.7.4. Entrapment efficacy

The CQE phytosome were centrifuged and the supernatant was scanned at a wavelength of 220 nm using UV-vis spectroscopy to assess the free CQE and the entrapment efficacy was calculated using the following formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug} - \text{Amount of free drug}}{\text{Total amount of drug}} \text{X } 100$$

The formulation trials were performed in triplicate and the outcomes are displayed as ±SD.

5.7.5. *In-vitro* diffusion study

In the diffusion study for 14 h in a modified Franz diffusion cell apparatus using a dialysis membrane, the percentage of diffusion was plotted on a graph. The trials were run three times and the results were shown as ±SD. Based on the results the optimized CQE phytosomes were selected and further studies were carried out. The release kinetics of the optimized formulation were calculated by the mathematical models such as zero-order kinetics, first-order kinetics, Higuchi kinetic, Kors Meyer–Peppa's model, and Hixson–Crowell cube root law and the r^2 was noted. Physicochemical and *in-vitro* diffusion study revealed that the formulation F2 is an optimized one. Further studies were continued with F2 formulation.

5.7.6. Anti-oxidant activity for the optimized cqe phytosomes (f2)

5.7.6.1. DPPH assay

Standard ascorbic acid and phytosome were made at different concentrations separately. DPPH were combined and after 30 minutes of dark incubation at room temperature, the samples were examined in a UV spectrophotometer at 517 nm. A graph was developed once the scavenging activity was estimated.

5.7.6.2. Nitric oxide scavenging

The standard ascorbic acid and phytosome concentrations (100, 200, 400, 800, and 1000 µg/mL) were made separately and mixed with sodium nitroprusside (10 mM in phosphate-

buffered saline) and incubated for 4 hours at 37°C. After the incubation period, 0.5 mL of Griess reagent was added and the absorbance at 546 nm was determined. The graph was drawn.

5.7.7. Anti-inflammatory activity for the CQE phytosomes f2

Several assays were used to assess the phytosomal formulation's anti-inflammatory properties. Using spectrophotometric data, the protein denaturation assay evaluated the inhibition of protein denaturation and calculated the percentage of inhibition. Trypsin and casein substrates were used to evaluate the proteinase inhibitory activity and calculate the percentage inhibition of proteinase activity. In addition, a mixture of buffer, lipoxygenase, and extract was used in the lipoxygenase inhibition assay; absorbance measurements were used to calculate the percentage of inhibition. All of these tests together gave rise to a thorough assessment of the extract's anti-inflammatory potential.

5.7.8. Skin irritation studies

From the above studies, the best formulation (F2) was selected for skin irritation testing. The skin irritation test was carried out in Cape Bio Lab & Research Centre, Tamil Nadu, following approval from the Institutional Animal Ethical Committee (IAEC) (PU/CAHF/25th IAEC/2020/02) under CPCSEA certification The experiment protocol for the CQE phytosomes has been approved by the institutional animal ethical committee and conducted according to the guidance of Indian Natural Science Academy for the use and care of experimental animals. The test was carried out for 21 days and observed for erythema and edema after the 7th, 14th, and 21st days.

5.8. Preparation of transdermal patches

The blank patches and the *Cissus quandragularis* phytosome-loaded patches were prepared using the solvent evaporation method by incorporating polymers in different ratios (Table 2). The patches were stored for further studies.

Table 1: Trial Formulations of Phytosome-loaded Transdermal patches

Ingredients	**Formulation code**			
	F1	**F2**	**F3**	**F4**
CQ phytosomes (mg)	100	100	100	100
HPMC K15M (mg)	100	200	300	400
Polyethylene glycol 4000 (mg)	0.1	0.1	0.1	0.1
Dimethyl sulfoxide (mL)	0.1	0.1	0.1	0.1

Ethanol (mL)	5	5	5	5
Distilled water (mL)	5	5	5	5

5.9. Evaluation of transdermal patches [15]

The evaluation of transdermal patches involved visual inspection, weight uniformity, thickness uniformity, folding endurance, moisture content, moisture uptake, and CQE content uniformity. The patches underwent a 24-hour exposure to humidity, drying, folding, and weighing. By dissolving a patch in a solvent and utilizing UV spectrophotometry to measure the concentration, the CQE content was determined. To ensure accuracy and standard deviation, each analysis was carried out three times. The results are displayed as average values with (±SD).

5.9.1. *In-vitro* diffusion study

The *in-vitro* diffusion study of the transdermal patches was carried out using a modified Franz diffusion cell. The patch was placed in the donor compartment and the receptor compartment was filled with phosphate buffer (pH 7.4). The samples were withdrawn at predetermined time intervals and analyzed for CQE content using UV spectrophotometry at 220 nm. Based on this study, the optimized formulation was selected and the release kinetics of the optimized formulation were calculated by the mathematical models such as zero-order kinetics, first-order kinetics, Higuchi kinetic, Kors Meyer–Peppa's model, and Hixson–Crowell cube root law and the r^2 was noted.

5.10. Stability study for the optimized phytosome-loaded transdermal patch

The stability study for the optimized formulation was carried out by ICH guidelines (acceleration study for 40°C ± 2°C RH 75% and room temperature) for three months, later it was evaluated for visual appearance, shape, pH, leakage, odour, CQE content, and *in-vitro* CQE diffusion study.

5.11. *In-vivo* study for the optimized phytosome-loaded transdermal patch

For in-vivo evaluation (Table 3), healthy male and female *Wistar albino* rats weighing 150–200 g were obtained from Cape Bio Lab & Research Centre, Tamil Nadu, following approval from the Institutional Animal Ethical Committee (IAEC) under CPCSEA certification (PU/CAHF/25th IAEC/2020/02). Rats were housed in well-ventilated cages with a 12-hour

light and dark cycle, provided a standard pellet diet and had ad libitum access to water. Before experiments, animals were fasted overnight with continued access to water.

Table 2: Experimental design for *in-vivo* study of phytosome-loaded transdermal patch

S. No.	Study and method	Species	Gender	Body weight (g)	No. of animals
1.	Anti-arthritic and Carrageenan induced method	AWR	M&F	150–250	24
2.	Acute toxicity study	AWR	F	150–250	9
	Total	**AWR**	**M&F**	**150–250**	**33**

5.11.1. Acute toxicity for the optimized phytosome-loaded transdermal patch

Acute toxicity was assessed by OECD guidelines using *Wistar albino* rats, where a single oral dose of 2000 mg/kg body weight of the test extract was administered. This dose, known to be non-lethal based on previous studies, was monitored closely over 24 hours post-administration and observations continued for 14 days to record any adverse effects or mortality.

5.11.2. The anti-arthritic activity for the optimized phytosome-loaded transdermal patch

The anti-arthritis activity of *Cissus quadrangularis* phytosomes transdermal patches was investigated using albino Wistar rats. Arthritis was induced via intra-articular injection of carrageenan solution into the left hind paw, following which the rats were divided into experimental groups. These included a control group, a group administered with marketed transdermal patches, a group with *Cissus quadrangularis* phytosomes transdermal patches and an arthritis control group. Paw inflammation was measured using a Plethysmometer over 15 days, with results indicating a significant reduction in oedema volume compared to the arthritis control group (***$P < 0.001$). This study underscores the potential therapeutic efficacy of *Cissus quadrangularis* phytosomes in managing arthritis-induced inflammation [16].

5.11.3. Statistical analysis

All experiments were conducted in triplicate the results were expressed as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's

multiple comparison tests to determine significant differences between the groups. A *p*-value of < 0.05 was considered statistically significant.

6. RESULTS AND DISCUSSION

6.1. Microscopy and macroscopy of the *Cissus quadrangularis* stem

The stem of Cissus quandragularis exhibits a rectangular outline with discontinuous vascular bundle rings (Figure 2). The epidermis is single-layered with actinocytic stomata encircled by small cells, forming a girdle-like sheath. Cortex cells are thin-walled, containing chloroplasts, starch grains, and calcium oxalate raphides. Vascular bundles are sclerenchymatous-capped, surrounded by collenchymatous arcs, with a central pith and four to six rows of phloem cells around xylem vessels. These compounds are crucial for the management of arthritis, as they can potentially reduce inflammation and promote bone healing by providing essential nutrients and active substances directly to the affected areas. These findings corroborate the already reported study [17].

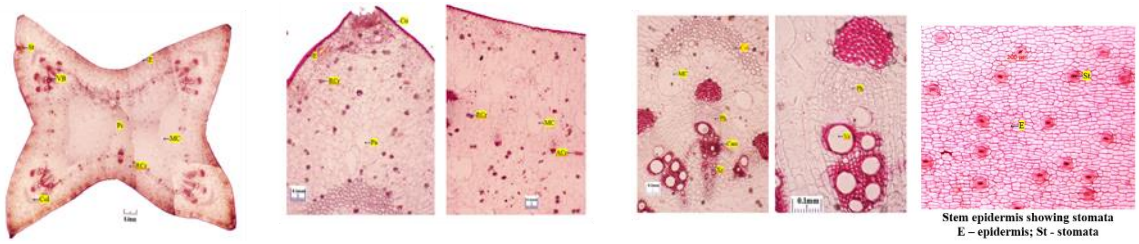


Figure 2: Microscopy of *Cissus quandragularis* Linn stem

6.2. Extraction and characterization

Crude ethanolic extract from *C. quadrangularis* was obtained via Soxhlet extraction. The extract exhibited a wet weight of 10 g and a dry weight of 4.5 g per 500 g of crude CQE, with a dark brownish-yellow colour. This concentrated extract is rich in bioactive compounds

6.2.1. Phytochemical analysis

Phytochemical screening of the ethanolic extract revealed (Table 4) the presence of alkaloids, phenolic compounds, amino acids, saponins, and flavonoids and the absence of carbohydrates. Some plant extracts were reported in devoid of carbohydrates. [18]

The presence of steroids and sterols is of greater importance as it is the major bioactive compound which is responsible for the anti-arthritis activity. Moreover, the phenolic compound exhibits anti-oxidant activity which alleviates the pharmacological activity of the CQE.

Table 3: Phytochemical screening by conventional qualitative method

S. No.	Phytochemicals	Presence/absence
1	Alkaloids	+
2	Carbohydrate	−
3	Glycosides	+
4	Phenolic compounds	+
5	Protein and amino acid	+
6	Gum and mucilage	+
7	Flavones and flavonoids	+
8	Saponins	+
9	Steroids and sterols	+
10	Tannins	+

6.2.2. UV spectroscopy

The observed peaks are at specific wavelengths which indicates the presence of conjugated or aromatic structures in the sample (Figure 3). The observed significant absorption peaks at various wavelengths strongly suggest the presence of aromatic or conjugated structures within the sample. A study reported the absorption maxima of *Cissus quadrangularis* extract at 221 nm, 271.50 nm, and 649.50 nm similar to this present study. [19] The absorption maxima of CQE at 220 nm in the present study corresponds to β-sitosterol which is the major phytoconstituent.

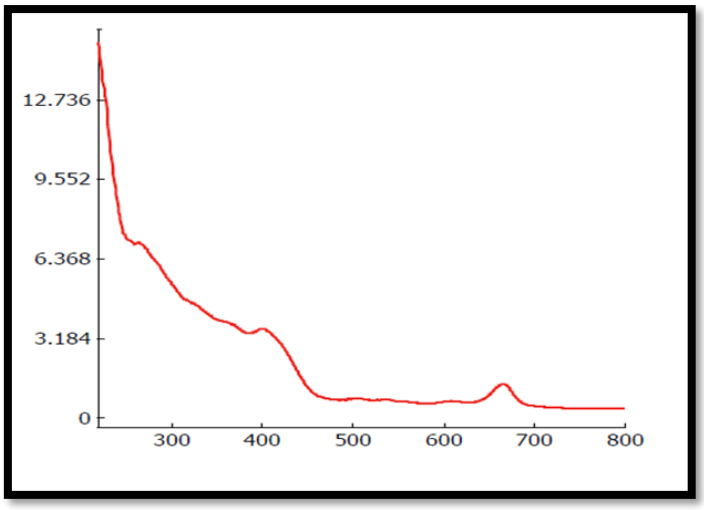


Figure 3: UV spectroscopy of ethanolic extract of *Cissus quadrangularis*

6.2.3. TLC

Flavonoids (Fig.4a), alkaloids (Fig.4b), tannins (Fig.4c) and phenols (Fig.4d) are present in the TLC analysis, with Rf values of 0.56, 0.41, 0.23, and 0.78 (Figure 4 and Table 5). When compared to the standards, these results demonstrate that the extracts retain their phytoconstituents. According to Asad Ullah et al., 2021,[20]. Phytoconstituents with bone-healing and anti-inflammatory qualities, such as flavonoids and phenols, can reduce inflammation and stimulate the formation of collagen, which can help manage the symptoms of arthritis and improve joint health.

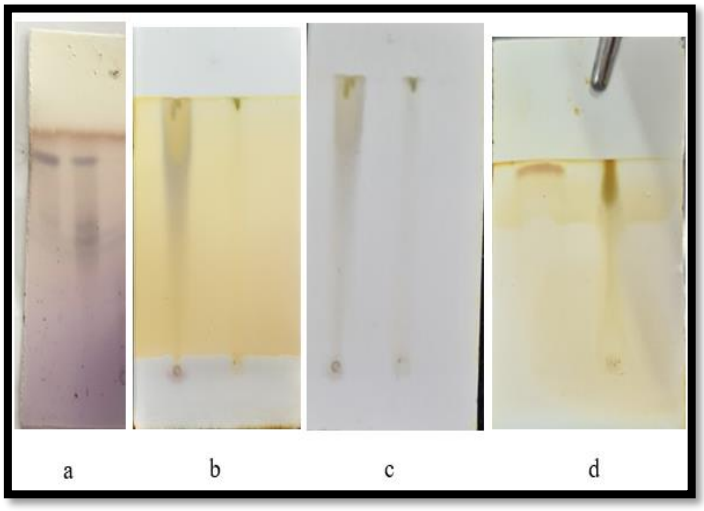


Figure 4: TLC of *Cissus quadrangularis*

Table 4: Details of phytochemical analysis by TLC

S. No.	Phytochemical test	Standard	Mobile phase	Rf value
1	Flavonoids	Quercetin	n-Butanol:Ethyl acetate:Water (1:2:3)	0.56
2	Alkaloids	Quinine	Ethyl acetate:Chloroform:Water (5:3:1)	0.41
3	Phenols	Gallic acid	Methanol:Water (2:1)	0.78
4	Tannins	Gallic acid	Chloroform:Water (3:2)	0.23

6.2.4. LC-MS

With a molecular weight of 448.4, quercetin-3-O-alpha-L-rhamnopyranoside and beta-sitosterol are the most abundant ingredients in the extract compared to the other chemicals Qian et al 2022, [21] reported that compounds like beta-amyrone can enhance bone regeneration and repair, aiding in the recovery of bone damage often seen in arthritis patients. The presence of sterol in the extract was confirmed by LC-MS. Kaur et al [19] reported the LC-MS profiling of the ethanolic extract of *Cissus quadrangularis* which contains similar compounds as our study. Figure 5 and Table 6 list the compound identified by LC-MS and Figure 6 shows the chromatogram of LC-MS.

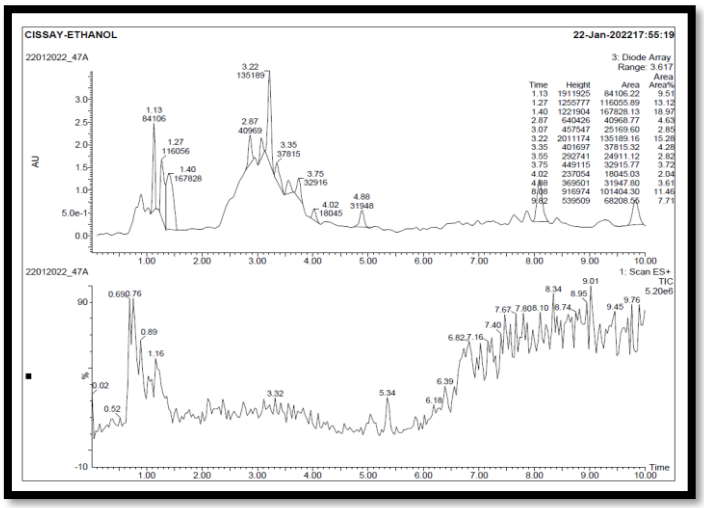


Figure 5: Chromatogram of LC-MS

Table 5: Details of phytochemical contents by LC-MS

S. No.	Compound name	Molecular weight
1	Quercetin-3-O-alpha-L-rhamnopyranoside	448.4
2	Vanilline	152.36
3	Beta-amyrone	424.43
4	Beta-sitosterol acetate	456.73
5	Cholesterin	385.42
6	Quinoline	129.16
7	Phytol	295.86
8	Erucic acid	338.20
9	Hexadecanoic acid	258.24
10	Carotene	535.59
11	Linoleic acid	280.13
12	Vaccenic acid	282.29
13	Oleic acid, 10-octadecenoic acid	282.30
14	Tetradecanoic acid	228.31
15	Pentadecanoic acid	242.29
16	Isopropyl stearate	326.83

6.2.5. GC-MS

β-Sitosterol, 4-ethylbenzoic acid and antra-9,10-quinone-compounds with high mass (M/z) values in the analysis were among those found by GC-MS. Thus, the presence of fatty acids and steroids demonstrates that these are necessary to comprehend the possible uses of the extracts in arthritis. A study reported the presence of fatty acids and aromatics in the extract of CS whose GC-MS data showed the highest presence of saturated and unsaturated fatty acids [22]. Table 7 shows the GC-MS reports and Figure 6 displays the chromatogram of GC-MS.

Table 6: Details of phytochemical screening by GC-MS

Phytoconstituents	Mass (M/z)
B-Sitosterol	414
4-Ethylbenzoic acid	150

Antra-9,10-quinone	343
2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	207
1,4-Phthalazinedione, 2,3-dihydro-6-nitro	207
5-Methyl-2-phenylindolizine	207
2-Nitro-4-(trifluoromethyl)phenol	207
Octanoic acid	298
Vitamin E	431.50

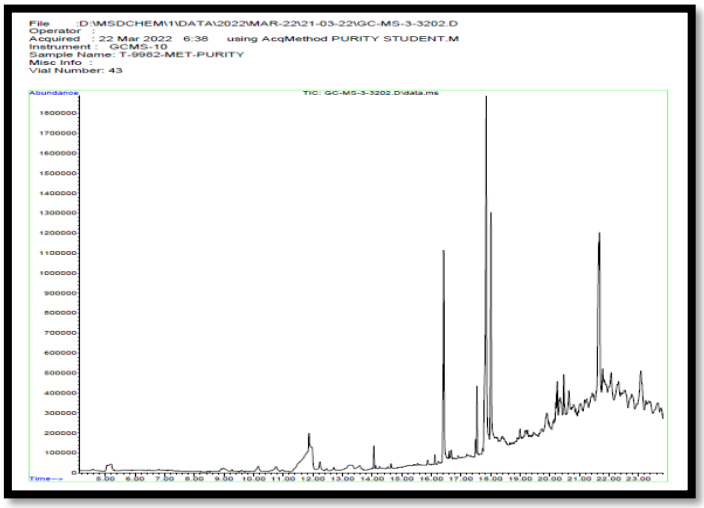


Figure 6: Chromatogram of GC-MS

6.2.6. HPLC

The HPLC analysis of the ethanolic extract of Cissus quadrangularis revealed a detailed chromatographic profile characterized by distinct peaks identified by their retention times (RT), corresponding heights (µV), and relative area percentages. Noteworthy peaks were observed at RT values of 1.646, 9.645, 10.501, and 15.539 minutes, each contributing differently to the overall composition as indicated by their area percentages. Additionally, the peaks at RT values of 16.935 and 17.156 minutes suggest the presence of novel compounds, as these unique retention times and chromatographic characteristics point to unidentified constituents within the extract. The chromatogram underscores the complexity of the extract,

with peaks displaying a range of intensities, as reflected by the height (µV) values. This comprehensive HPLC analysis offers valuable insights into the composition of the C. quadrangularis extract, setting a foundation for further exploration and identification of novel compounds contributing to the plant's bioactive profile, as illustrated in Fig 7

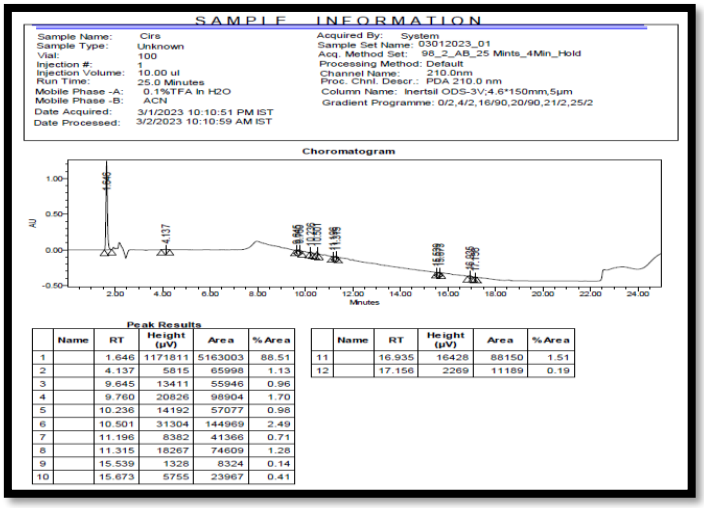

SAMPLE INFORMATION
Sample Name: Cirs
Sample Type: Unknown
Vial: 100
Injection #: 1
Injection Volume: 10.00 ul
Run Time: 25.0 Minutes
Mobile Phase -A: 0.1%TFA In H2O
Mobile Phase -B: ACN
Date Acquired: 3/1/2023 10:10:51 PM IST
Date Processed: 3/2/2023 10:10:59 AM IST
Acquired By: System
Sample Set Name: 03012023_01
Acq. Method Set: 98_2_AB_25 Mints_4Min_Hold
Processing Method: Default
Channel Name: 210.0nm
Proc. Chnl. Descr.: PDA 210.0 nm
Column Name: Inertsil ODS-3V;4.6*150mm,5µm
Gradient Programme: 0/2,4/2,16/90,20/90,21/2,25/2
Chromatogram
AU
Minutes
Peak Results
Name RT Height (µV) Area % Area
1 1.646 1171811 5163003 88.51
2 4.137 5815 65998 1.13
3 9.645 13411 55946 0.96
4 9.760 20826 98904 1.70
5 10.236 14192 57077 0.98
6 10.501 31304 144969 2.49
7 11.196 8382 41366 0.71
8 11.315 18267 74609 1.28
9 15.539 1328 8324 0.14
10 15.673 5755 23967 0.41
11 16.935 16428 88150 1.51
12 17.156 2269 11189 0.19

Figure 7: Chromatogram of HPLC

6.2.7. Molecular docking[MD]

Using molecular docking (Table 8 and Figure 8), it is possible to determine the binding affinities of beta-amyrone, beta-sitosterol acetate, cholesterin, and prednisolone with various receptors, indicating potential therapeutic applications for the extract in the treatment of inflammations. The binding of beta-sitosterol to inflammatory receptors can inhibit the production of pro-inflammatory cytokines, reducing arthritis symptoms was reported by Qian et al 2022 [21]. Similarly, the interaction of cholesterin with bone-related receptors can enhance bone formation and repair processes by kim eta al., 2020 [23]

Table 7: Binding efficiency of bioactive compounds

Ligand	**Binding affinity (kcal/mol)**
6RMJ-M_Beta-amyrone	−6.5
6RMJ-M_Beta-sitosterolacetate	−5.9
6RMJ-M_Cholesterin	−6.1
6RMJ-M_Prednisolone	−6.1
1CVU-M_Beta-amyrone	−8.2

1CVU-M_Beta-sitosterolacetate	−7.4
1CVU-M_Cholesterin	−8.4
1CVU-M_Prednisolone	−8.3

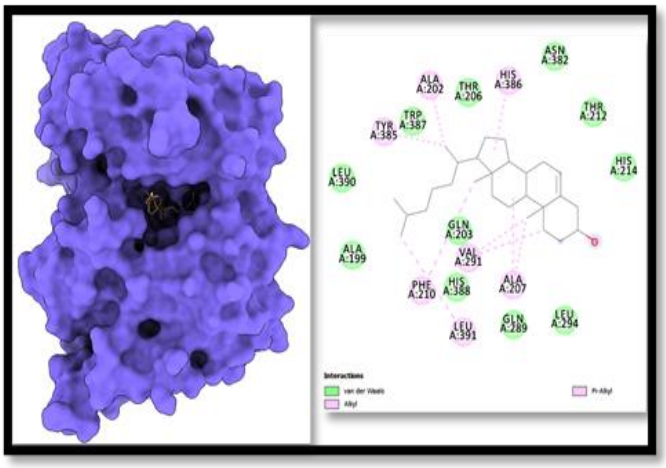


Figure 8: Surface view of the optimal pose of 1CVU complexed with cholesterin

6.2.8. MD simulation analysis

With its Cα backbone, RMSD, RMSF, Rg, and hydrogen bonding, 1CVU-cholesterin reveals its structural flexibility, stability, compactness, and intermolecular interactions, indicating its dynamic behaviour and functional ramifications (Figure 9). The MD study validated the presence of steroids which was confirmed by the above experiments.

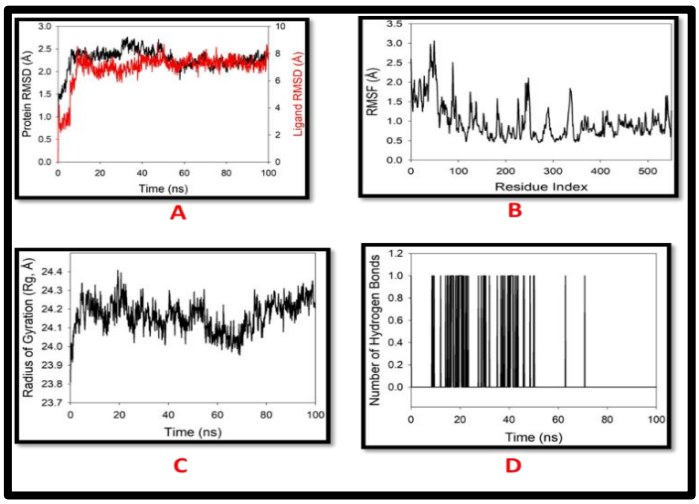


Figure 9: A. RMSD (root mean square deviation), B. RMSF (root mean square fluctuation), C. Radius of gyration, D. Simulation trajectory

6.2.9. Stability

The cholesterin's highest affinity exhibits exceptional stability, as seen by its compact structure, which is indicated by consistent Rg values. Additionally, the complex's persistent hydrogen bond interaction demonstrates its structural integrity and importance.

6.3. Evaluation of phytosomes

Table 9 presents the process parameters and different formulations used in the optimization of phytosomes based on vesicle formation. UV-vis spectroscopy confirmed that the prepared phytosomes had a distinctive peak at 220 nm (Figure 10). This encapsulation is crucial for preserving and effectively delivering the bioactive compounds, particularly beta-sitosterol, which are known for their anti-inflammatory properties. These properties help reduce inflammation and oxidative stress in arthritic joints, thereby managing arthritis and promoting bone health. The particle size analysis revealed an average size of 86.29 nm and a Zeta potential of −20.9 (Figure 11), enhancing the bioavailability and stability of the phytosomes, which is essential for effective drug delivery and the repair of bone tissue in arthritic conditions. Light microscopy confirmed the presence of well-formed vesicles (Figure 12), indicative of successful phytosome formulation necessary for the controlled release of bioactive compounds, thereby providing continuous anti-inflammatory effects and supporting bone healing. TEM and SEM studies revealed discrete vesicular structures and the disappearance of crystalline forms, which are crucial for improved drug delivery and skin permeation. The X-RD analysis, showed crystalline peaks at particular 2θ angles (Figure 13) while comparing with the results of Deleanu et al. [24]. This stability and sustained release ensure prolonged therapeutic effects, crucial for continuous reduction of inflammation and bone regeneration. TEM validated the nanoscale morphology of the phytosomes (Figure 14a), essential for maintaining structural integrity and effective distribution of bioactive compounds, maximizing the therapeutic effects of CQE. FE-SEM revealed a consistent distribution of nanoparticles (Figure 14b), improving stability and encapsulation, which is vital for reliable therapeutic outcomes in managing arthritis and promoting bone healing. EDAX determined the essential elemental composition (Figure 15), confirming the presence of elements like calcium and phosphorus, essential for bone health and regeneration paralleling the findings from Kaur et al. [25]. The FT-IR spectrum (Figure 16) of the final optimized CQE phytosome (F2) reveals characteristic peaks that confirm the successful encapsulation of CQE within the phytosome, indicating the preservation of key functional groups essential for its biological activity.

Table 8: Optimization of CQE phytosome formulation

Formulation code	**CQE (CQE): LP30 ratio**	**Process temperature (°C)**	**Revolutions per minute (RPM)**	**Sonication temperature (°C)**	**Size of vesicles**	**Sonication time (min)**	**Observation**
F1	01:04	Below 60°C	50 ± 5	5 C to 10°C	Size of vesicles decreases	10	Incomplete and large sized vesicles
F2	01:05	60°C ± 2°C	100 ± 5	10°C to 20°C	Numerous vesicles of moderate size	20	Spherical vesicles
F3	01:06	80°C ± 2°C	>100	Room temperature	Large size vesicles	30	Broken vesicles

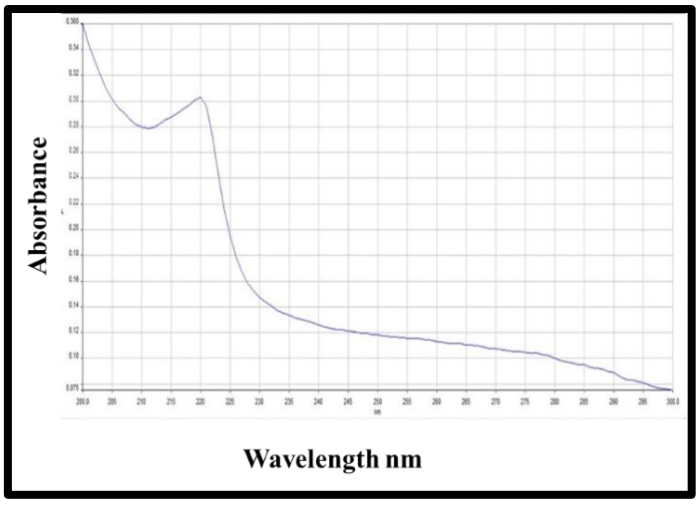


Figure 10: UV-visible spectra of final optimized CQE phytosome F2

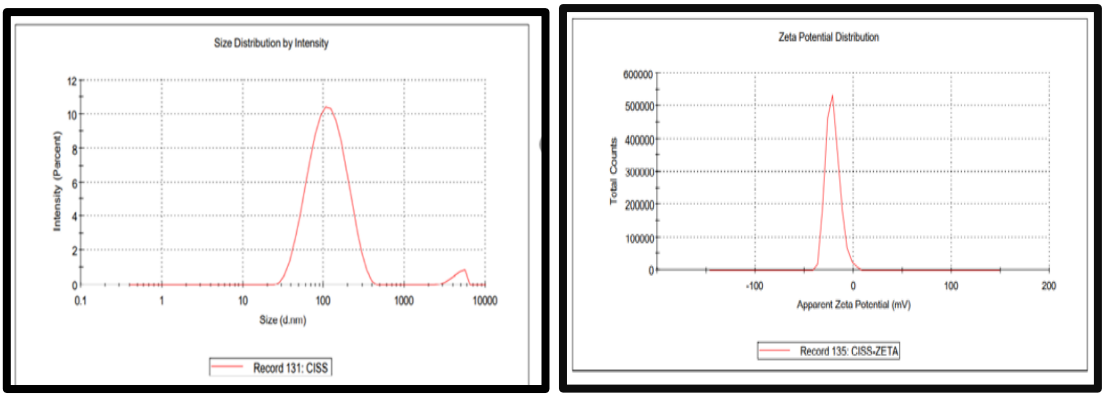


Figure 11: Particle size and surface charge analysis of final optimized CQE phytosome F2

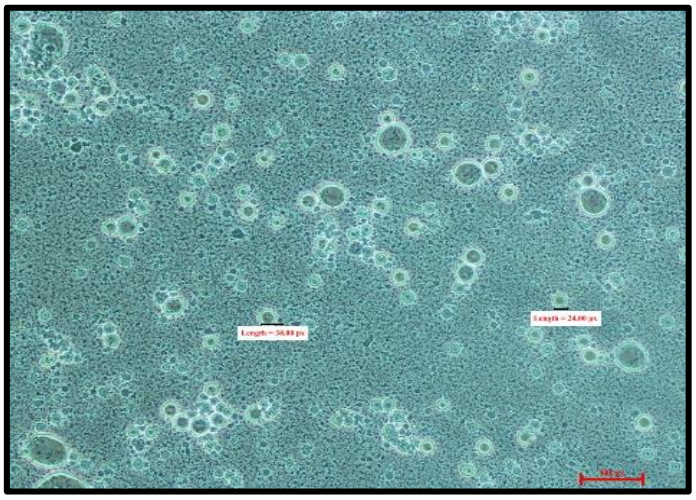


Figure 12: Light microscope of final optimized CQE phytosome F2

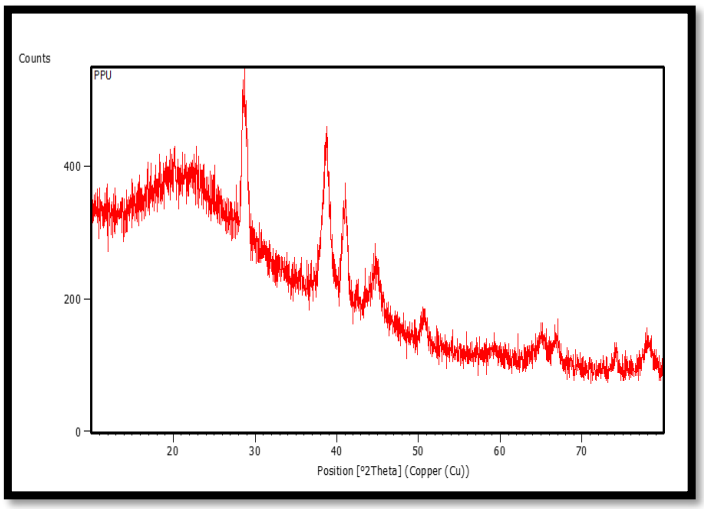
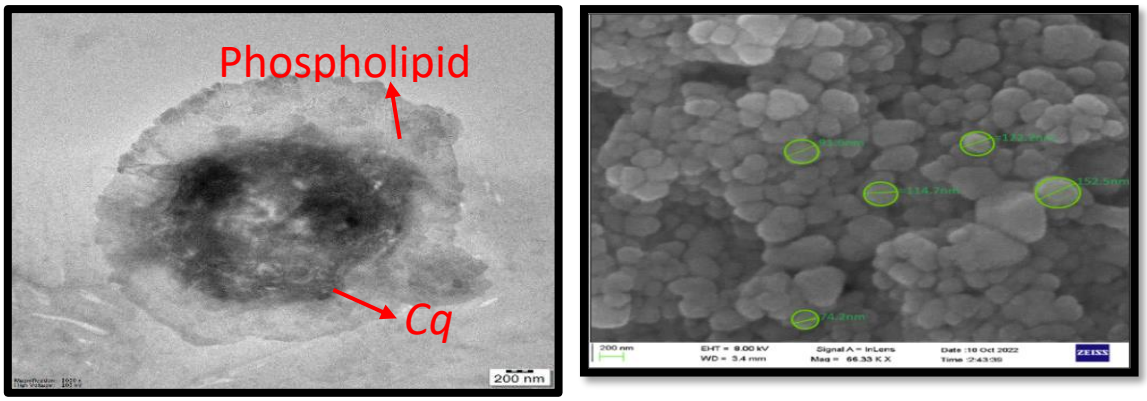


Figure 13: XRD spectra of final optimized CQE phytosome F2



a b

Figure 14: TEM and SEM analysis of final optimized CQE phytosomes (F2)

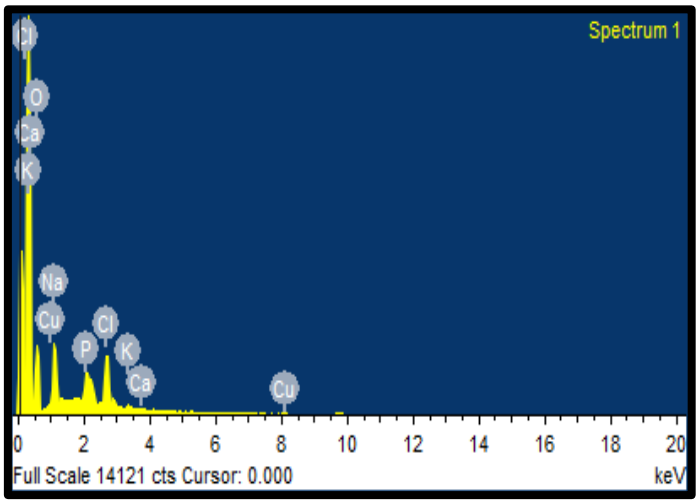


Figure 15: EDAX of final optimized CQE phytosome F2

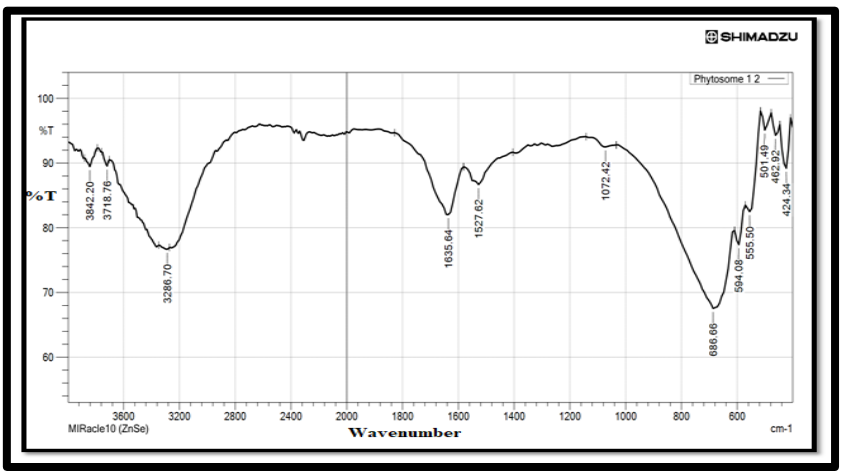


Figure 16: FT-IR spectra of final optimized CQE phytosome F2

6.3.1. UV-Vis spectroscopy scanning

The sample was analyzed using a UV-Vis spectrophotometer within the wavelength range of 200-800 nm. The absorbance spectrum was recorded, with a particular focus on detecting peaks around 220 nm to identify the characteristic peak of beta-sitosterol (Figure 17). According to Wei et al. (2010) [26], beta-sitosterol is soluble in ethanol, and Shah et al. (2010) [27] reported the peak of beta-sitosterol at 202 nm. These findings provide a reference for interpreting the observed peaks in our analysis and confirm the presence of beta-sitosterol in the sample. Based on this calibration curve was determined.

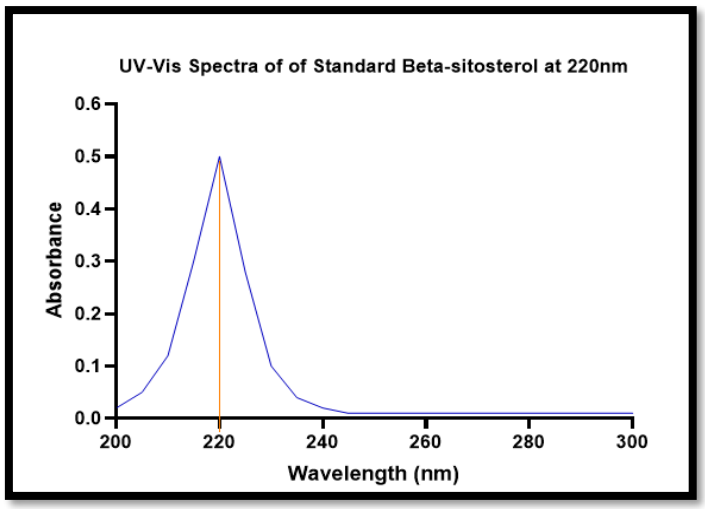


Figure 17: UV- Vis Spectra of Standard Beta-sitosterol at 220nm

6.3.2. Calibration Curve for the standard Beta-sitosterol

A calibration curve for beta-sitosterol at 220 nm was established by preparing a series of standard solutions in ethanol at concentrations of 0, 2, 4, 6, 8, 10, and 12 µg/mL. Each solution was measured at 220 nm using a UV-Vis spectrophotometer, and the absorbance values were recorded. A linear regression analysis was performed to create the calibration curve and R2 0.999 (Figure. 18). For the determination of *Cissus quadrangularis* phytosome's unknown concentration, an accurately measured sample was dissolved in ethanol and its absorbance at 220 nm was recorded. The unknown concentration was calculated by comparing the absorbance of the sample to the established calibration curve, using the linear equation derived from the standard solutions.

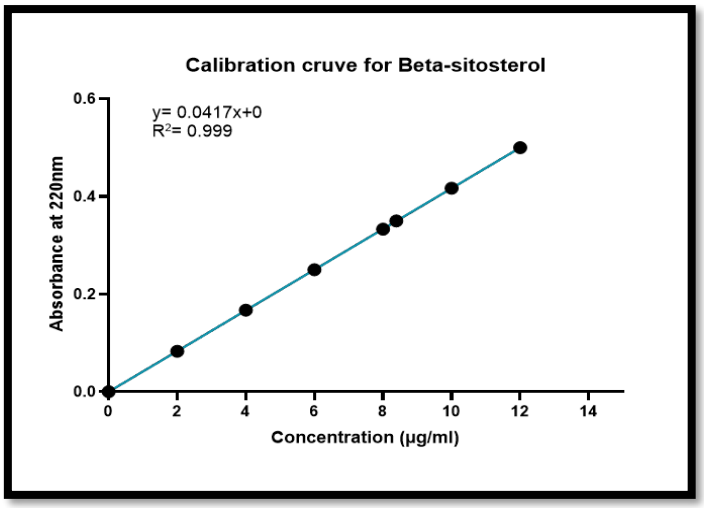


Figure 18:Calibration Curve for the standard Beta-sitosterol at 220nm

6.3.3. Calibration curve

The UV spectrophotometric analysis of *Cissus quandragularis* phytosomes (Figure 19) showed a high correlation coefficient ($R^2 = 0.9996$) in the calibration curve, validating its accuracy in quantifying concentrations from 2 to 10 µg/mL, essential for precise formulation and dosage determination.

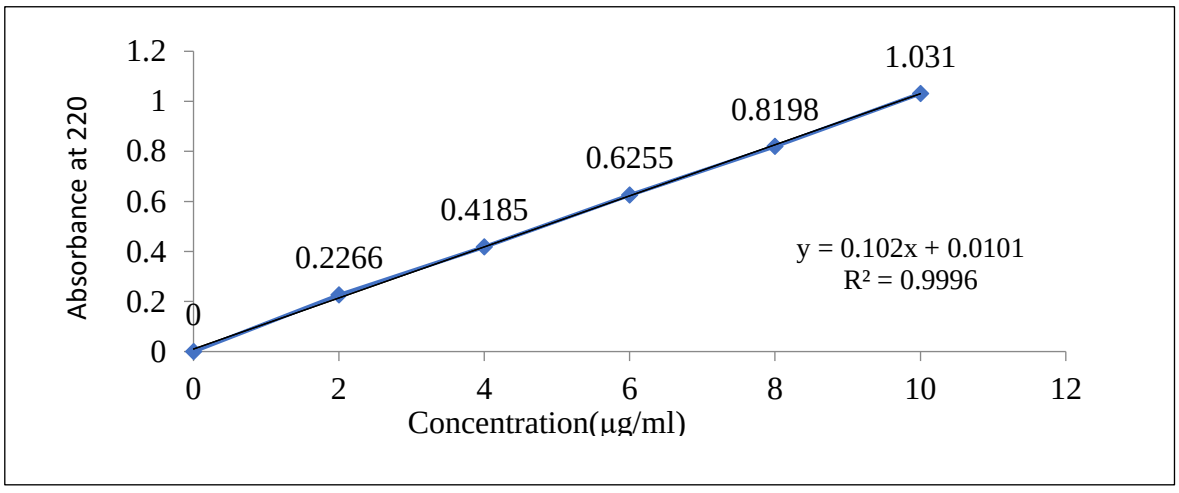


Figure 19:Calibration curve of CQE *Cissus quadrangularis*

6.3.4. FT-IR analysis

FT-IR spectra (Figure 20) revealed distinct peaks for both the *Cissus quadrangularis* extract and phospholipids. The peaks of extract seen at 3302 cm^{-1} for -OH stretching vibration, 2905 cm^{-1} for -CH stretching vibration, 1643 cm^{-1} for -c=o stretching vibration, 1527 cm^{-1} for -NH bending vibration, 1388 cm^{-1} -CH3 for surface bending vibration, 1080 cm^{-1} for -c-o-c

stretching vibration, 666 cm^{-1} for -CH bends. This analysis provides critical insights into the chemical compatibility and stability of the formulation. Some peaks of extract not evidenced in the CSE loaded phytosomes, and peak shift near 3302 cm^{-1} represents the bonding between extract and components of phytosomes which is similar to the study reported by Nandini et al. [28]. The bond formed is hydrogen bond which will cleave and release the active constitutes when exposed to body fluids to body fluids to produce its pharmacological action.

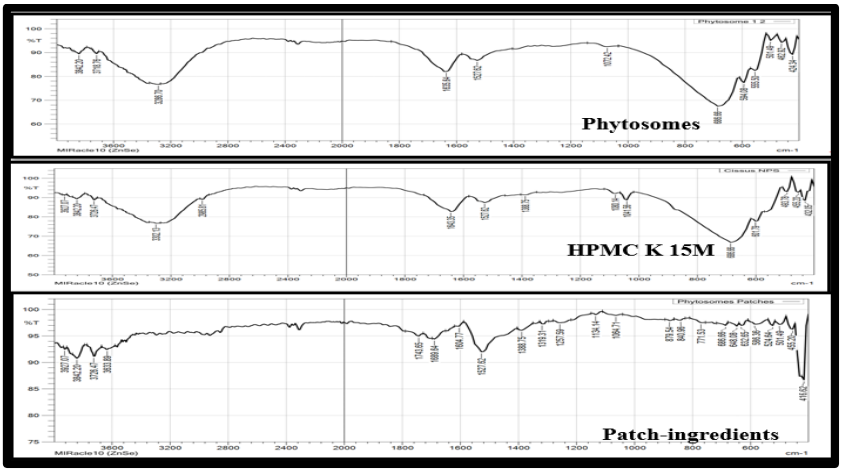


Figure 20: Comparative FT IR spectra

6.3.5. DSC analysis

The DSC analysis reveals the peak that represents the moisture loss (Figure 21). The produced phytosomes show an endothermic peak at 102°C, which indicates that lecithin has integrated the extract into the lipid layer. The lack of additional peaks verifies that the extract has been loaded into the lipid layer. This study was comparable to how lecithin's addition to the lipid matrix (LM) greatly affects the behavior of crystallization, as demonstrated by the WAXD and thermoanalysis was evaluated by Schubert et al., 2005 [29]. The peaks disappeared, confirming that the encapsulation was effective. The components of the patch increased thermal stability, resulting in a melting point measurement of 127°C, which is higher than the melting temperatures of PEG and HPMC.

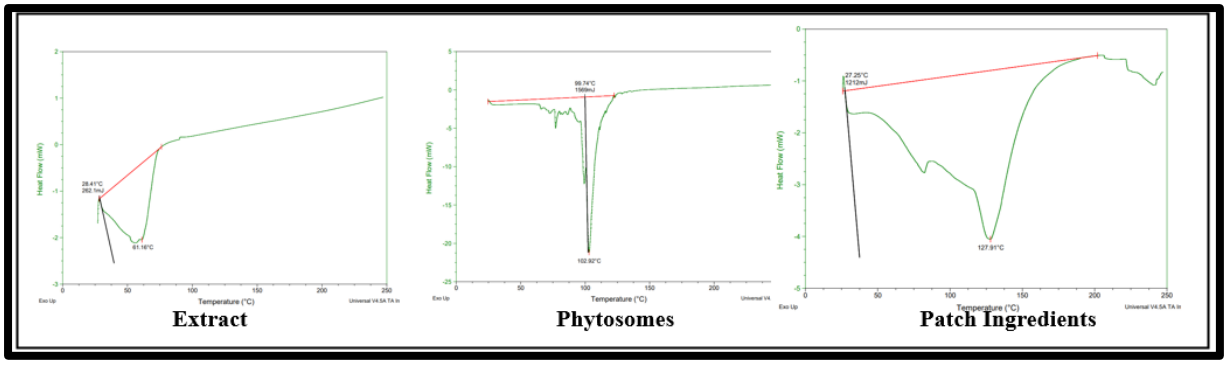


Figure 21: Comparative DSC thermogram

6.3.6. CQE content and entrapment efficiency

High CQE content (86.84% ± 0.84%) and entrapment efficiency (86.38% ± 0.92%) indicate effective encapsulation of *Cissus quadrangularis* bioactives within the phytosome structure, ensuring consistent dosage delivery and therapeutic efficacy. High EE produced was suggested due to the lipid/drug ratio and process condition used for CQE phytosome preparations. Because of the specific nature of CQ, it can intercalate into the ordered structure of phosphatidyl choline effectively, without drug leakage. The lipid composition of phytosome membrane components influences the efficacy of entrapped drugs and the formed vesicular system is efficient to increase its ability to hold the hydrophobic extract until it reaches the specific site to produce its biological action.

6.3.7. *In-vitro* CQE diffusion study

The in-vitro diffusion study of the CQE phytosomes over 12 hours, F2 was released at percentage of 83.62 ± 1.58 (Figure 22). As a result, F2 was chosen as the optimal phytosome trial. The optimized trial F2 release kinetics was carried out following the mathematical model based on the findings (Table 9). The Higuchi model's high r^2 value suggests that a diffusion-controlled mechanism governs CQE release. This view is supported by the similarly high r^2 value for the Korsmeyer–Peppas model, which also sheds light on the characteristics of the diffusion process. As a result, in line with Fickian diffusion, the CQE release mostly occurs via a diffusion-controlled release mechanism, most likely from a matrix system. The release mechanism is suggested to be a combined effect of diffusion and surface erosion which corroborate the reported study [30].

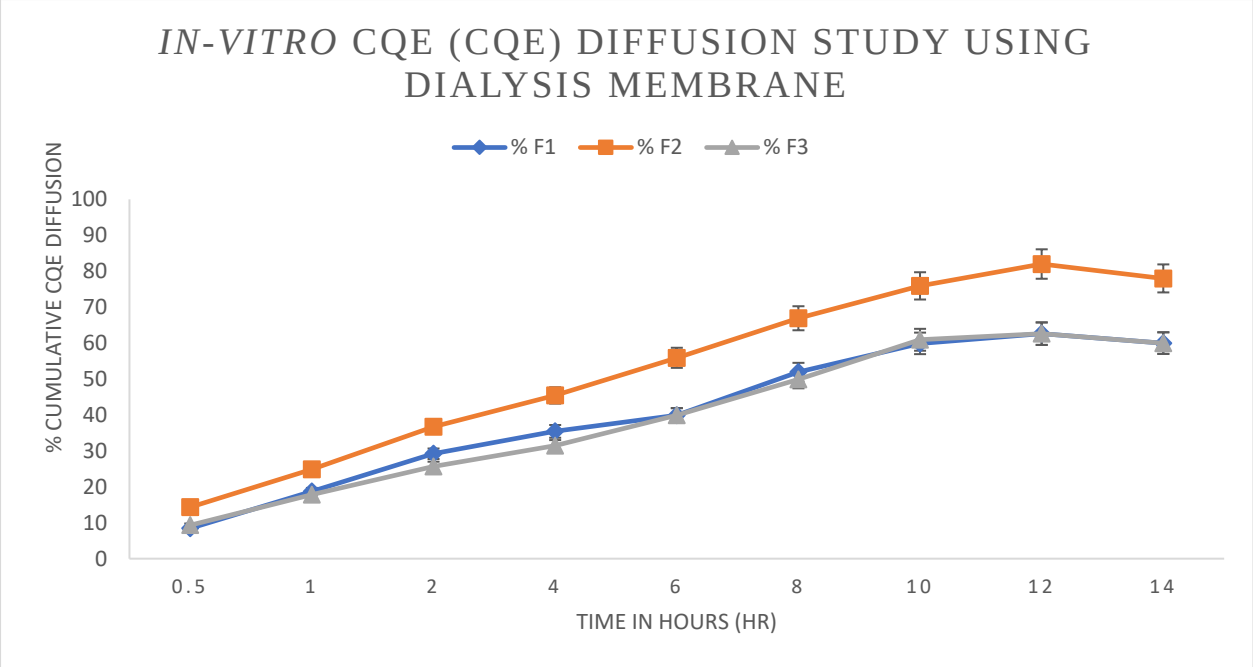


Figure 22*: In-vitro* CQE diffusion of transdermal patches

Table 9:Release kinetics of the optimized formulation F2

Model	**Zero order**	**First order**	**Higuchi**	**Korsmeyer–Peppas**
r^2	0.5835	0.9158	0.9773	0.9805

6.3.8. Antioxidant activity

7.3.6.1. DPPH assay

The DPPH assay confirmed the potent antioxidant activity of CQE phytosomes, showing dose-dependent inhibition (Figure 23) of free radicals comparable to standard antioxidants. This highlights its potential in combating oxidative stress-related disorders.

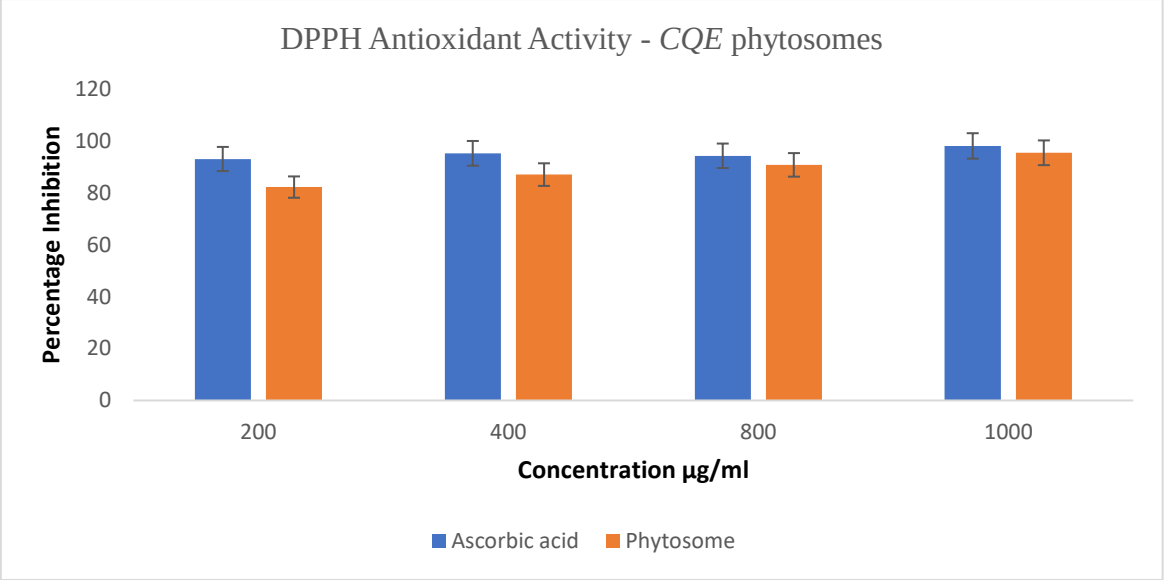


Figure 23: DPPH assay for the CQE phytosomes

7.3.6.2. Nitric oxide scavenging activity

The nitric oxide scavenging assay demonstrated significant inhibition by CQE phytosomes (Figure 24), with increasing activity from 70.89% ± 0.02% to 98.59% ± 0.01% across concentrations from 100 to 1000 µg/mL. These findings underscore its potential therapeutic utility in oxidative stress management.

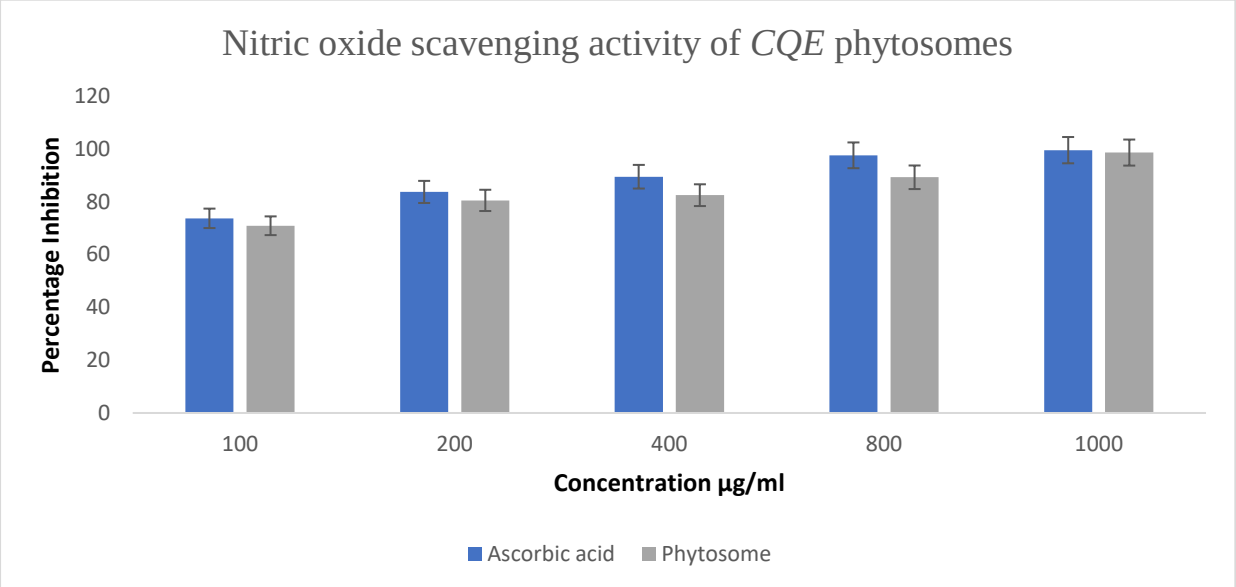


Figure 24: Nitric oxide scavenging activity of CQE phytosomes

The polyphenols found in CQE are what give it the ability to scavenge free radicals, making it an antioxidant. It can transfer an electron from the -OH groups connected to the phenolic

rings, along with a hydrogen nucleus, to a free radical. The free radical is stabilized and rendered inactive by this electron. The antioxidant activity of compound is important as it synergises the other biological activities [31].

6.3.9. Anti-inflammatory assay for CQE phytosomes

Employing the biochemical assays, CQE phytosome ability to reduce inflammation was assessed. The extract strongly prevented protein denaturation in a dose-dependent manner, as demonstrated by the protein denaturation assay, which showed an increase from 72.5% ± 0.05% at 100 µg/mL to 96.3% ± 0.12% at 1000 µg/mL (Figure 25A). This characteristic is advantageous for inflammatory illnesses and bone health. Furthermore, the proteinase inhibition assay showed that the extract inhibited protease activity in a concentration-dependent manner, with inhibition levels ranging from 65.7% ± 0.42% to 94.7% ± 0.04%. This indicates that the CQE phytosomes may have the ability to reduce inflammatory conditions, which are important for preserving bone health (Figure 25B). Additionally, the lipoxygenase inhibition experiment demonstrated dose-dependent inhibition (Figure 25C). The denaturation of proteins corresponds to proteins losing their secondary, tertiary, and quaternary structures, resulting in loss of biological function. The high anti-inflammatory activity of CQE phytosomes was confirmed by the inhibition of protein denaturation and similar studies was reported in a study [32]

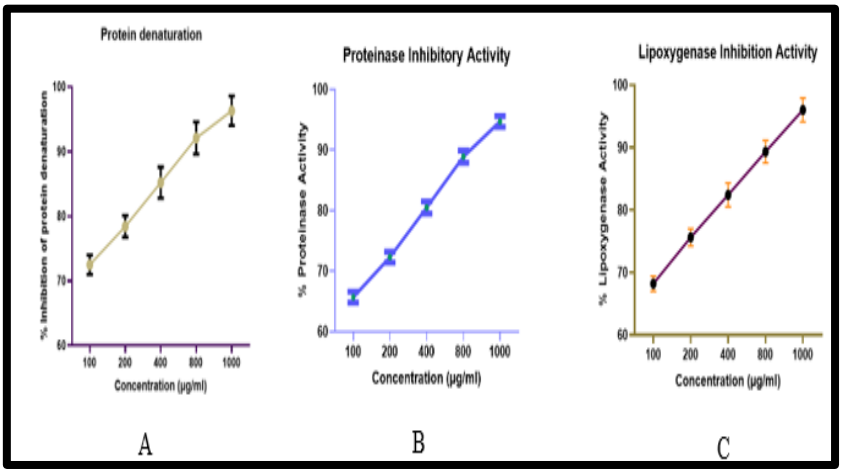


Figure 25: Anti-Inflammatory assay of CQE phytosomes

6.3.10. *In-vivo* skin irritation study for CQE phytosomes f2

By assessing the grading of skin irritation over 7, 14, and 21 days, the skin irritation study sought to determine the safety and tolerability of various formulations, including a control

carbopol gel (Group 1), a plain CQ extract (Group 2), and CQE phytosome F2 (Group 3) was in Table 10.

Table 10: Grading of skin irritation study of CQE phytosome formulation F2

Name of the group	Animal numbers	Grading		
		7th day	14th day	21st day
	1	0	0	0
	2	0	0	0
Group 1	3	0	0	0
(carbopol gel)	4	0	0	0
	5	0	0	0
	6	0	0	0
	7	2	2	2
	8	3	3	3
Group 2	9	2	2	2
(psoralen in gel)	10	3	3	3
	11	2	2	3
	12	2	2	2
	13	0	1	1
	14	0	0	0
Group 3	15	1	1	1
(CQE phytosome F2)	16	0	0	0
	17	0	0	1
	18	1	1	1

0 – No erythema, 1 – minimally perceptible erythema, 2 – marked erythema, 3 – Fiery red erythema with edema

In Group 3, CQE phytosomes F2 showed almost no skin irritation or minimal erythema, where as in Group 2, plain CQE showed marked erythema and fiery red erythema with edema. The Group 1 carbopol gel base showed no erythema and edema. Hence, this study proves the biocompatibility of the CQE phytosomes which is the important properties of a drug delivery system.

6.4. Physicochemical evaluation of phytosome-loaded transdermal patch (results are tabulated in table 11)

When evaluating the transdermal patches containing phytosomes of *Cissus quadrangularis*, formulation F2 outperformed the others in several crucial areas (Table 11). F2 demonstrated ideal consistency and controllable dimensions for application, with a weight of 0.45 ± 0.74 mg and a thickness of 0.53 ± 0.34 mm. Additionally, F2 showed 251% ± 0.22% folding endurance,

demonstrating outstanding mechanical strength and flexibility – two critical factors for the long-term viability of transdermal patches. Furthermore, F2 exhibited the lowest moisture content (3.63% ± 0.23%) and moisture uptake (2.85% ± 1.03%), indicating improved stability and a lower chance of microbial contamination. In addition, the formulation demonstrated a noteworthy percent elongation of 94% ± 1.18%, indicating its potential elasticity and flexibility in response to changes in the skin. Moreover, the surface pH of 7.5 ± 0.72 agrees with the pH of natural skin, reducing the likelihood of irritation. Above all, F2 had the greatest CQE concentration (86.87% ± 0.62%), supporting efficient active ingredient delivery. All of these characteristics integrate to make F2 the best formulation possible. It exhibits a well-balanced mix of mechanical strength, stability, and bioactive content, which makes it the most promising option for therapeutic applications. The similar results were reported in a study [28]

Table 11: Physicochemical evaluation of phytosome loaded transdermal patches

Formulation code	**Uniformity of weight (mg)**	**Thickness (mm)**	**Folding endurance (%)**	**Moisture uptake (%)**	**Moisture content (%)**	**Percent elongation (%)**	**Surface pH**	**CQE (CQE) content (%)**
F1	0.60 ± 0.25	0.70 ± 1.40	150 ± 1.00	5.50 ± 0.10	6.20 ± 0.20	65 ± 1.20	6.5 ± 0.80	72.50 ± 1.00
F2	0.45 ± 0.74	0.53 ± 0.34	251 ± 0.22	2.85 ± 1.03	3.63 ± 0.23	94 ± 1.18	7.5 ± 0.72	86.87 ± 0.62
F3	0.75 ± 0.60	0.80 ± 0.25	140 ± 1.20	6.00 ± 0.80	7.10 ± 0.40	55 ± 1.00	6.0 ± 0.90	68.30 ± 1.50
F4	0.65 ± 0.50	0.75 ± 0.30	160 ± 1.10	5.80 ± 0.70	6.80 ± 0.50	60 ± 1.10	6.2 ± 0.85	70.40 ± 1.20

6.4.1. CQE diffusion studies

The study shows a gradual release profile over 12 hours, with formulations F2 consistently achieving the highest cumulative CQE diffusion (78.00% ± 1.20) and in 14 hours (76.00 ± 1.10), followed by F1, F4, and F3 (Figure 26). Standard deviations indicate the data uniformly across formulations, highlighting F2's superior performance in sustained CQE release. The release kinetics show that the Korsmeyer–Peppas model's (Table 12) best fit suggests a controlled release mechanism involving both diffusion and erosion. In general, the drug from the transdermal patch initially penetrates through the stratum corneum and then passes through

the deeper epidermis and dermis without drug accumulation in the dermal layer. When the drug reaches the dermal layer, it becomes available for systemic absorption via dermal microcirculation [33]. Moreover, this sustained CQE release profile aligns with therapeutic needs, promising prolonged efficacy and patient compliance in transdermal CQE delivery systems.

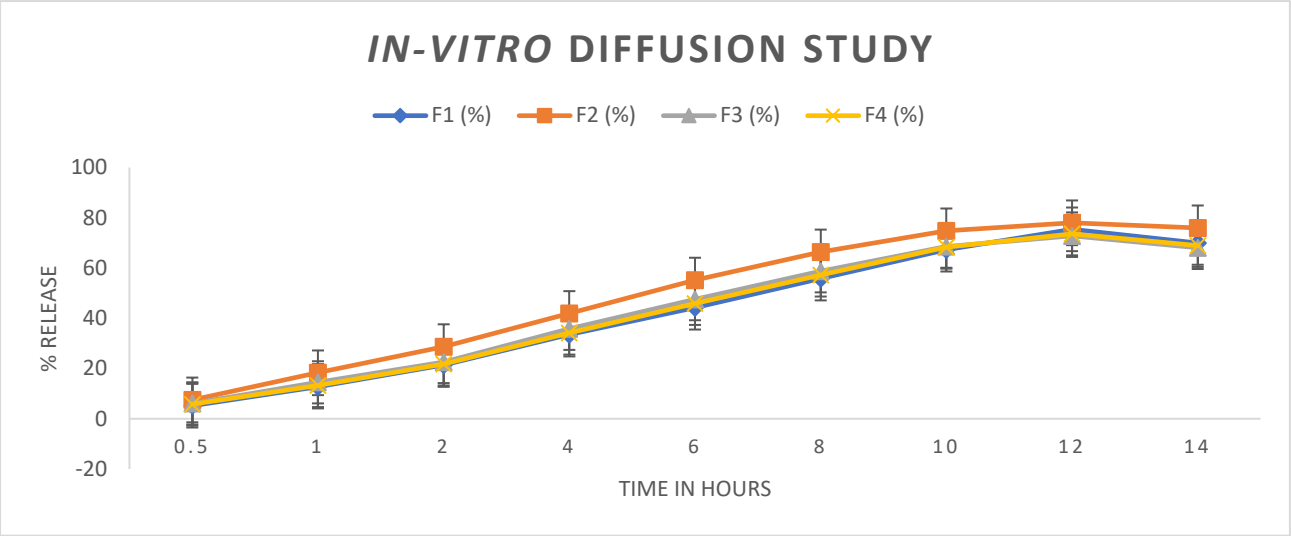


Figure 26: *In-vitro* diffusion of phytosome loaded transdermal patches

Table 12: Mathematical model of release kinetics of the optimized phytosome loaded transdermal patches

Models	**Zero-order kinetics**	**First-order kinetics**	**Higuchi model**	**Korsmeyer–Peppas model**
r^2	0.9100	0.9908	0.9687	0.9729

6.5. Stability Studies

The stability study for the optimized F2 was performed based on the ICH guideline, the results are reported in Table 13 and an in-vitro CQE diffusion study was done in Figure 27 shows the percentage of release after three months of study.

Table 13: Stability studies of the optimized phytosome loaded transdermal patch (F2)

S. No.	Parameters	40°C ± 2°C RH 75%				Room temperature			
		Initial	**At the end of first month**	**At the end of second month**	**At the end of third month**	**Initial**	**At the end of first month**	**At the end of second month**	**At the end of third month**
1.	Visual appearance	Cloudy	Cloudy	Cloudy	Cloudy	Cloudy	Cloudy	Cloudy	Cloudy
2.	Shape	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical
3.	pH	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
5.	Leakage	Not found	Not found	Not found	Not found	Not found	Not found	Not found	Not found
6.	Odor	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless
7	CQE content	86.87 ± 0.62	86.07 ± 0.71	85.83 ± 1.25	85.85 ± 1.23	86.87 ± 0.62	85.27 ± 1.78	85.01 ± 0.22	84.98 ± 0.32

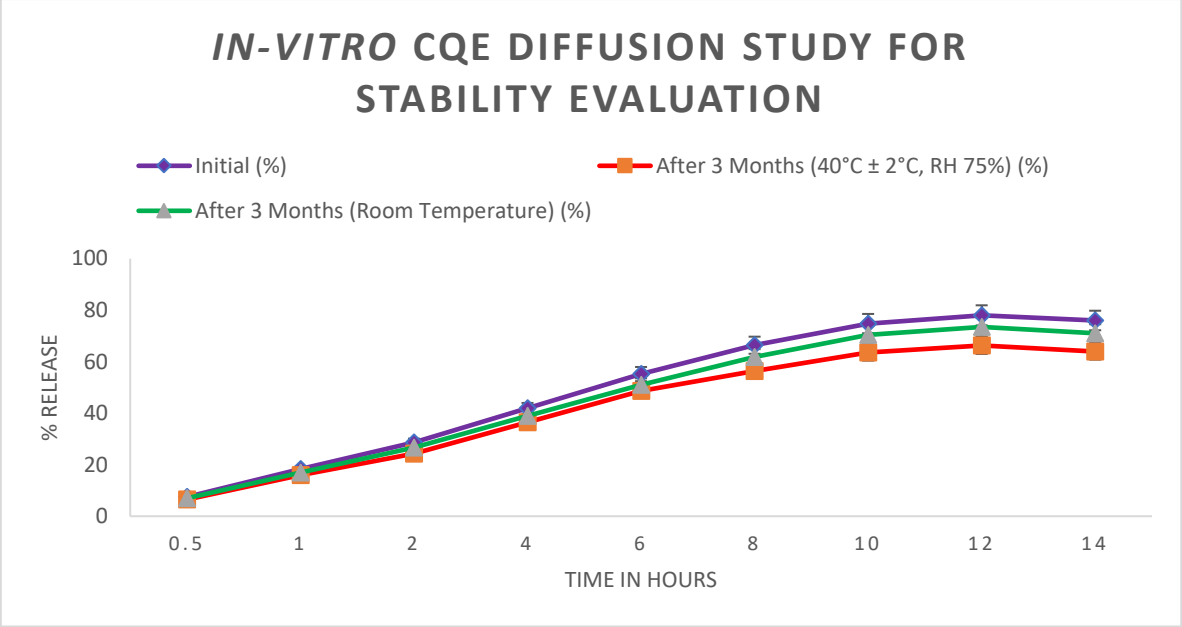


Figure 27: In-vitro CQE diffusion study for stability evaluation

6.6. *In-vivo* evaluation – acute toxicity

The absence of acute toxicity symptoms in female rats indicates the safety profile of phytosome loaded transdermal patch (F2) (Figure 28). Normal physiological parameters post-administration support their potential for safe therapeutic use, encouraging further investigations into long-term safety and efficacy.

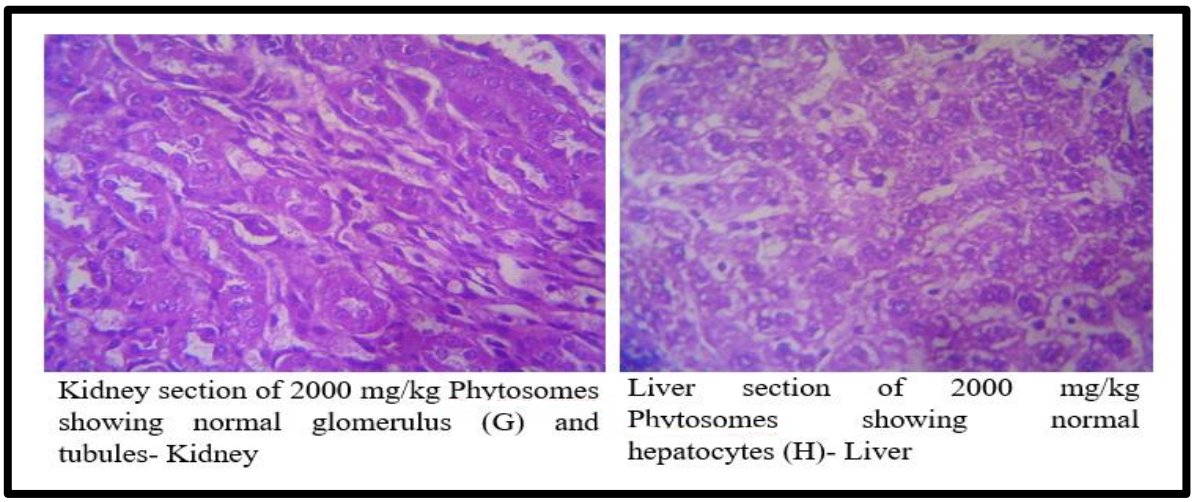

Kidney section of 2000 mg/kg Phytosomes showing normal glomerulus (G) and tubules- Kidney
Liver section of 2000 mg/kg Phytosomes showing normal hepatocytes (H)- Liver

Figure 28: Acute toxicity study for phytosome-loaded transdermal patch of F2 formulation

6.7. Measurement of paw thickness

The DDA transdermal patch outperformed Volini Gel, which had a peak reduction of 65.57% at 6 hours but fell to 36.18% at 24 hours, according to Sharma et al. (2022) [34]. The DDA

transdermal patch significantly reduced rat paw edema with a 63.81% inhibition at 24 hours. In a similar vein, (F2) showed (Figure 29) that it significantly decreased the thickness of the paws in arthritic rats, suggesting that this natural alternative may be a promising treatment for arthritis. Additional clinical validation is necessary to validate these results.

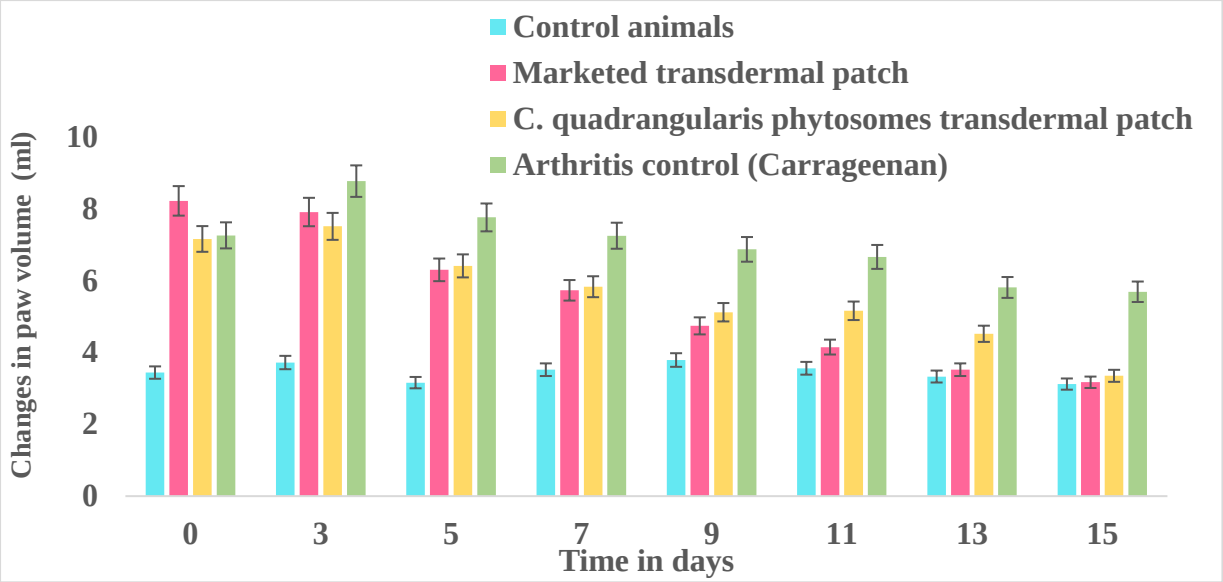


Figure 29: Evaluation of anti-arthritis activity of phytosome loaded transdermal patch – F2 by paw volume method

6.8. Evaluation of anti-arthritis activity – histopathology of bone at the joints

Histopathological findings reveal potential anti-inflammatory effects of phytosome-loaded transdermal patch (F2) on joint tissues (Figure 30). Preservation of normal bone structure and inflammatory response modulation suggest their therapeutic potential in mitigating arthritis-induced changes, advocating for deeper mechanistic studies.

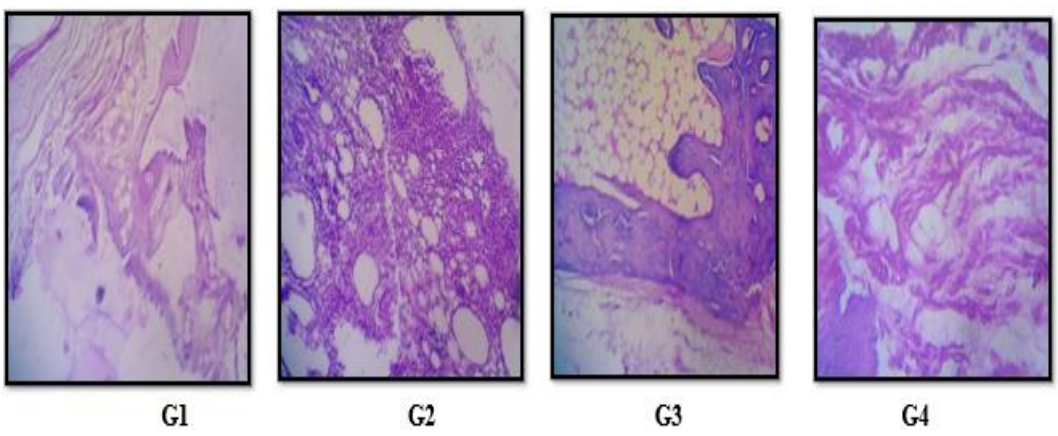


Figure 30: Histopathology of bone at the joints

6.8.1. Mechanism of anti-inflammatory action

As *Cissus quadrangularis* Linn. (CQ) has anti-inflammatory molecular mechanisms, creating a unique transdermal patch of CQ for the treatment of arthritis and promotion of bone repair is a viable therapeutic approach. The anti-arthritic and therapeutic benefits of the traditional medicinal herb *Cissus quadrangularis* on bone health have been the subject of much research. With the transdermal delivery method, bioactive chemicals can be released into the afflicted area in a regulated and non-invasive manner, resulting in decreased systemic adverse effects and increased bioavailability. Phytosterols, which have strong anti-inflammatory effects, are among the bioactive substances found in CQ, along with triterpenes and flavonoids. By modifying important inflammatory pathways, these substances lower the expression of pro-inflammatory cytokines such TNF-α, IL-1β, and IL-6. CQ successfully lowers the transcription of these cytokines, which lessens the inflammatory response linked to arthritis by blocking the NF-κB signaling pathway [35]. Furthermore, CQ functions as a dual-action drug, increasing bone repair and lowering inflammation by suppressing osteoclastogenesis and enhancing osteoblast proliferation and differentiation. For those who suffer from arthritis, where long-term inflammation frequently results in bone loss, this is especially advantageous. By using a transdermal formulation, these active ingredients are guaranteed to effectively cross the skin's barrier and reach the joints and bones in therapeutic concentrations. Additionally, a transdermal patch containing CQ offers prolonged release, sustaining steady therapeutic levels for a longer amount of time. Compared to oral formulations, which need frequent administration and may cause gastrointestinal side effects, this can result in higher patient compliance.

7. SUMMARY AND CONCLUSION

Macro and microscopy analysis verified the structural characteristics of *Cissus quadrangularis*. Ethanolic extraction produced 10 g of wet and 4.5 g of dry crude extract, yielding 2% and 0.9%, respectively. Bioactive compounds, including alkaloids, flavonoids, amino acids, carbohydrates, saponins, tannins, and phenolic compounds, were identified. TLC, LC-MS, and GC-MS revealed the specific phytochemicals, including flavonoids, alkaloids, phenols, tannins, Quercetin-3-O-alpha-L-rhamnopyranoside, vanillin, beta-amyrone, fatty acids, beta-sitosterol, 4-ethylbenzoic acid, and Vitamin E. Detailed chromatographic profile indicated the presence of novel compounds. In silico analysis, they represented the compounds with potential anti-inflammatory and antirheumatic properties. The identified compounds suggested potential

applications in cardiovascular health, antimicrobial activities, antioxidant processes and other pharmacological effects.

The particle size of the CQE phytosome is ideal for enhancing bioavailability and facilitating the delivery of bioactive compounds. The interaction studies indicated the presence of aromatic or conjugated structures, molecular composition and functional groups of bioactive components in the phytosomes. X-ray diffraction analysis provided insights into the crystalline nature, influencing stability and CQE phytosome release kinetics. The appreciable sustained CQE phytosome release demonstrated the favourable characteristics of CQE phytosome for pharmaceutical applications. HR-TEM and FE-SEM confirmed nanoscale spherical morphology and it also shows anti-inflammatory activity. The skin irritation and acute toxicity study in adult male rabbits/female rats showed that *Cissus quandragularis* phytosomes are safe, with no adverse effects. The phytosome-loaded transdermal patches significantly reduced skin/mucous membrane irritation and the paw volume and thickness suggesting protective effects on bone morphology.

In conclusion, *Cissus quadrangularis* Linn.'s innovative transdermal formulation uses its anti-inflammatory molecular pathways to offer a focused, effective, and patient-friendly method of treating arthritis and encouraging bone mending. Patients with bone-related illnesses and chronic inflammatory problems may find their quality of life greatly improved by this novel delivery technology. Further studies and clinical trials are necessary to validate the safety, efficacy and translational potential of CQE phytosome.

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ANNEXURE

1. List of Publication

Kiruba Mohandoss /Afr.J.Bio.Sc.6(13)(2024). 1715-1735 ISSN: 2663-2187

https://doi.org/10.48047/AFJBS.6.13.2024. 1715-1735

African Journal of Biological Sciences

Carrageenan-Induced Arthritic Joint Inflammation in Rats: Evaluating the Anti-Arthritic Potential of Phytosomal *Cissus quadrangularis* Ethanolic Extract.

Kiruba Mohandoss,[1] Vijayan V,[2*] Hemalatha S[3]

[1]Department of Pharmaceutics, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU) Porur, Chennai, India.

[2]School of pharmacy, Sri Balaji Vidyapeeth University, Puducherry, India Porur, Chennai.

[3]Department of Pharmacognosy, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU) Porur, Chennai, India.

Corresponding author

Dr. Vijayan V

School of pharmacy,

Sri Balaji Vidyapeeth University,

Puducherry, India Porur, Chennai-116.

Email: vijayanv2@gmail.com

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Abstract

This research synthesizes, characterizes, and applies *Cissus quadrangularis* phytosomes to transdermal patches for arthritis. Phytosomes were prepared from lipoid P 30 and crude *C. quadrangularis* ethanolic extract and characterized by optical microscopy, SPR, ATR-FTIR, XRD, FE-SEM, HRTEM, and Zetasizer Nano ZS. The evaporating solvent created transdermal patches with different polymer-phytosome ratios. To ensure the safety of the phytosomal formulation, its acute toxicity was investigated. In Wistar Albino rats, carrageenan intradermally caused arthritis was used to test the anti-arthritis activity of transdermal patches. Four groups of rats were tested: control, commercial transdermal patch, *C. quadrangularis* phytosome patch, and arthritic control. The *C. quadrangularis* phytosome transdermal patch reduced inflammation compared to that in the arthritic control group. Statistical analysis was performed using Prism 5. This study suggests *C. quadrangularis* phytosome transdermal patches may be used to treat arthritis, paving the way for future research. Microscopic investigation revealed the phytosome structural shape, content, and delivery system applications. UV spectroscopy revealed 15.044 OD220, indicating the presence of aromatic or conjugated structures. FT-IR spectroscopy revealed O-H stretching vibrations at 3842.20 cm^-1 in bioactive compounds. XRD analysis showed a phytosome crystallinity of 49.67 %. Particle analysis indicated good stability and dispersion with 86.29 an average particle size and -20.9 mV zeta potential. FESEM and HR-TEM exhibited nanoscale homogeneity and morphology. In acute toxicity testing of female rats, phytosomes were harmless. The phytosome transdermal patch reduced the paw volume and thickness of Wistar rats, similar to commercial anti-arthritis medications. Histopathological examination confirmed the anti-inflammatory effects of transdermal patches. Statistical analysis demonstrated significant improvements across the experimental groups, proving treatment efficacy. LC-MS revealed C. quadrangularis substances with anti-rheumatic properties. *C. quadrangularis* phytosomes may improve arthritis treatment via a holistic approach.

Keywords: *Cissus quadrangularis*, Ethanolic Extract, Phytosomes, FE-SEM and HR-TEM, Anti-Arthritic activity

African Journal of Biological Sciences

Research article

Comprehensive Phytochemical Profiling and Molecular Characterization of *Cissus quadrangularis* Ethanolic Extract: Towards Unveiling Therapeutic Potentials.

Kiruba Mohandoss,[1,2] Vijayan V,[2*] Hemalatha S[3]

[1]Department of Pharmaceutics, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU) and School of pharmacy, Sri Balaji Vidyapeeth University, Puducherry, India Porur, Chennai-116.

[2]School of Pharmacy, Sri Balaji Vidyapeeth University, Puducherry, India.

[3]Department of Pharmacognosy, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU) Porur, Chennai-116.

Corresponding author

Dr. Vijayan V, School of pharmacy,

Sri Balaji Vidyapeeth University, Puducherry, India Porur, Chennai-116. Email: vijayanv2@gmail.com

Article History

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Received: 18June 2024

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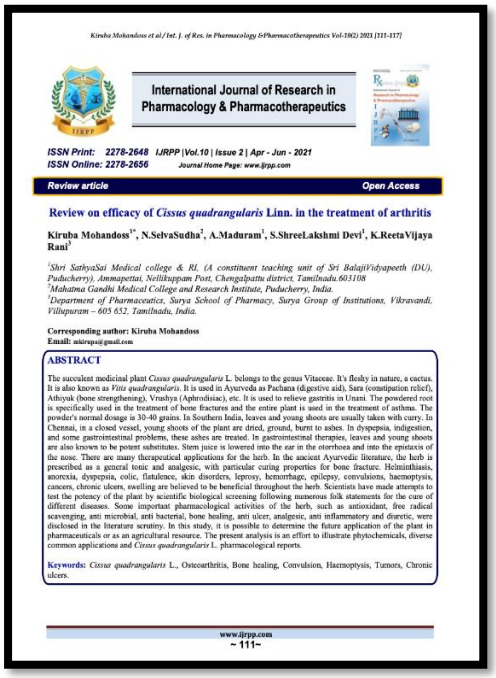
ABSTRACT

The stems of *Cissus quadrangularis* were collected systematically from the Coimbatore region of Tamil Nadu, India. Continuous ethanolic extraction using a Soxhlet apparatus yielded a crude ethanolic extract, which was subjected to percentage yield calculation for the extraction efficiency assessment. Phytochemical evaluation was performed using conventional thin-layer chromatography (TLC). Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses provided insights into the molecular constituents, whereas High-Performance Liquid Chromatography (HPLC) fingerprint analysis contributed to comprehensive characterization. The ethanolic extraction of *C. quadrangularis* using the Soxhlet technique resulted in a wet weight of the crude extract of 10 g, with a corresponding dry weight of 4.5 grams. The percentage yields calculated indicated that 2% of the wet ethanolic extract and 0.9% of the dry ethanolic extract were obtained from the crude drug. Phytochemical analysis revealed the presence of alkaloids, flavonoids, amino acids, carbohydrates, saponins, tannins, phenolic compounds, and more in the ethanolic extract, which is consistent with previous studies. Thin-layer chromatography (TLC) profiling further confirmed the presence of flavonoids, alkaloids, phenols, and tannins. LC-MS analysis identified a diverse array of compounds, including Quercetin-3-O-alpha-L-rhamnopyranoside, Vanilline, Beta-Amyrone, and various fatty acids, all of which have distinct pharmacological implications. The GC-MS analysis unveiled compounds such as B-Sitosterol, 4-Ethylbenzoic acid, and Vitamin E, contributing to the extract's potential health benefits. HPLC analysis provided a chromatographic profile indicating distinct peaks with varying intensities and area percentages, demonstrating the complexity of the *C. quadrangularis* ethanolic extract. This comprehensive characterization underscores the rich phytochemical diversity and potential bioactivity of this plant, paving the way for further exploration of its therapeutic applications.

Keywords: *Cissus quadrangularis*, Ethanolic extraction, Soxhlet extraction, LC-MS, Lyophilization.



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Review article Open Access

Review on efficacy of *Cissus quadrangularis* Linn. in the treatment of arthritis

Kiruba Mohandoss[1*], N.SelvaSudha[2], A.Maduram[1], S.ShreeLakshmi Devi[1], K.ReetaVijaya Rani[3]

[1]Shri SathyaSai Medical college & RI. (A constituent teaching unit of Sri BalajiVidyapeeth (DU). Puducherry), Ammapettai, Nellikuppam Post, Chengalpattu district, Tamilnadu.603108

[2]Mahatma Gandhi Medical College and Research Institute, Puducherry, India.

[3]Department of Pharmaceutics, Surya School of Pharmacy, Surya Group of Institutions, Vikravandi, Villupuram – 605 652, Tamilnadu, India.

Corresponding author: Kiruba Mohandoss

Email: ukirupa@gmail.com

ABSTRACT

The succulent medicinal plant *Cissus quadrangularis* L. belongs to the genus Vitaceae. It's fleshy in nature, a cactus. It is also known as *Vitis quadrangularis*. It is used in Ayurveda as Pachana (digestive aid), Sara (constipation relief), Athiyuk (bone strengthening), Vrushya (Aphrodisiac), etc. It is used to relieve gastritis in Unani. The powdered root is specifically used in the treatment of bone fractures and the entire plant is used in the treatment of asthma. The powder's normal dosage is 30-40 grains. In Southern India, leaves and young shoots are usually taken with curry. In Chennai, in a closed vessel, young shoots of the plant are dried, ground, burnt to ashes. In dyspepsia, indigestion, and some gastrointestinal problems, these ashes are treated. In gastrointestinal therapies, leaves and young shoots are also known to be potent substitutes. Stem juice is lowered into the ear in the otorrhoea and into the epistaxis of the nose. There are many therapeutical applications for the herb. In the ancient Ayurvedic literature, the herb is prescribed as a general tonic and analgesic, with particular curing properties for bone fracture. Helminthiasis, anorexia, dyspepsia, colic, flatulence, skin disorders, leprosy, hemorrhage, epilepsy, convulsions, haemoptysis, cancers, chronic ulcers, swelling are believed to be beneficial throughout the herb. Scientists have made attempts to test the potency of the plant by scientific biological screening following numerous folk statements for the cure of different diseases. Some important pharmacological activities of the herb, such as antioxidant, free radical scavenging, anti microbial, anti bacterial, bone healing, anti ulcer, analgesic, anti inflammatory and diuretic, were disclosed in the literature scrutiny. In this study, it is possible to determine the future application of the plant in pharmaceuticals or as an agricultural resource. The present analysis is an effort to illustrate phytochemicals, diverse common applications and *Cissus quadrangularis* L. pharmacological reports.

Keywords: *Cissus quadrangularis* L., Osteoarthritis, Bone healing, Convulsion, Haemoptysis, Tumors, Chronic ulcers.

2. Certification from the Siddha Institute for Authentication

சித்தமருத்துவ மைய ஆராய்ச்சி நிலையம்
(மத்திய சித்த மருத்துவ ஆராய்ச்சிக் குழுமம், ஆயுஷ் அமைச்சகம், இந்திய அரசு)
सिद्ध केंद्रीय अनुसन्धान संस्थान
(सी.सी.आर.एस., चेन्नई, आयुष मंत्रालय, भारत सरकार), अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई - 600106
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Anna Govt. Hospital Campus, Arumbakkam, Chennai – 600106, E-mail: crisiddha@gmail.com
Phone: 044-26214925, 26214809, Web: http://crisiddha.tn.nic.in

Form No. PCOG002-ARF

DEPARTMENT OF PHARMACOGNOSY
Analysis report for 238.C18032101R

Part A: Particulars of sample submitted

Test requested by: Kiruba M, PhD scholar, Shri Sathya Sai Medical College and Research Institute, Chengalpet 603108.
Requested on: 18.03.21
Investigation to be performed: Macroscopy, microscopy, quantitative microscopy and powder microscopy
Sample coded as: C18032101R
Sample details: Stem of *Cissus quadrangularis* L.

Part B: Methodology

1. **Macroscopy:** External feature of test sample was documented using Nikon COOLPIX 5400 digital camera.

2. **Microscopy***:* Sample was preserved in fixative FAA for more than 48 hr. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin. Transverse sections were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss Axio Cam Erc5s digital camera under bright field light. Magnifications were indicated by scale bar.

3. **Quantitative microscopy:** The fresh peel from the stem was boiled with 0.1% chloral hydrate solution and slides prepared were prepared. The stomatal number, stomatal index, and epidermal numbers were determined.

4. **Powder microscopy:** A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.

Part C: Result

1. Macroscopy of stem

Fresh stem is fleshy, glabrous, succulent, quadrangular with internodes measuring 7 to 21 cm in length and 1 to 2 cm in breadth, nodes constricted with attached leaf and tendril, woody and fibrous with four winged projections, pale greenish in colour (Figure 1) with characteristic odour and bitter taste.

Figure 1. Macroscopy of *Cissus quadrangularis* stem

3. Certification of IEAC for Animal Studies

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LL : 04651-270056
Mobile : +91 9994172256
+91 8381205610
E-mail: capebiolab@gmail.com
jaslinmpharm@gmail.com
Web: www.capebiolab.com

Dr. J. Jaslin Edward, M.Pharm.,Ph.D.
Managing Director

Date : 28/11/2022

Ref :
CBLRC/CLR/64/2022

INSTITUTIONAL ANIMAL ETHICAL COMMITTEE (IAEC)

CERTIFICATE

This is to certify that **MS. Kiruba Mohandoss,** Research fellow, Mahatma Gandhi Medical College and Research Institute Sri Balaji Vidyapeeth (sbv), Puducherry, has been granted approval by IAEC conducted on 26/11/2022 with approval **No. CBLRC/IAEC/10/02-2022** for the research work entitled **"Design and development of novel Transdermal formulation of *Cissus quadrangularis* Linn. for the management of arthritis and bone healing"** under the guidance of Dr. V.Vijayan, Professor, School of Pharmacy, Mahatma Gandhi Medical College and Research Institute Sri Balaji Vidyapeeth (SBV), Puducherry, India. for the award of Ph.D degree.

CAPE BIO LAB & RESEARCH CENTRE * Date : 28/11/2022

IAEC Chairman
Cape Bio Lab & Research Centre
Marthandam
K.K. Dist. - 629 165

Suggestion and Comments

Thank you for your valuable comments and Feedback